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ON THE INTERSTITIAL GRANULES AND FAT DROPLETS OF STRIATED MUSCLE

H. HAYS BULLARD

From the Anatomical Laboratory, Tulane University of Louisiana

SEVEN FIGURES

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The granules to be found between the myo-fibrils or muscle columns of cross striated muscle, although mentioned by Henle ('41) were first described in detail by Kölliker ('57) who called them 'interstitial granules.' He applied the term to both fat droplets and true interstitial granules, the latter being of a non-fatty nature. This paper presents observations concerning the structure of striated muscle with especial reference to interstitial granules and fat droplets, including also a brief discussion of their general occurrence, chemical nature, and physiological significance. A number of important communications dealing with the interstitial granules have included a somewhat comprehensive review of the literature, namely, Retzius ('90), Arnold ('09), Holmgren ('10), Prenant ('11), and Bell ('11). As several of these papers are of recent date I have thought best to omit a chronological review and shall discuss the literature only in so far as its subject matter has a direct bearing on the topics treated in this paper.

According to Kölliker both fat droplets and true interstitial granules are of wide distribution, occurring in vertebrate muscle and also in insect muscle. A few observers have denied the existence of two general types of interstitial granules, especially in vertebrate muscle, but usually the work of Kölliker has been confirmed in this respect. My observations are in accord with those of Kölliker and I shall likewise designate the types of granules as true interstitial granules and fat granules or fat droplets.

For the present it may be said that true interstitial granules, at least for the most part, are not completely soluble in absolute alcohol and not readily stained by fat stains such as Scharlach R, while the fat droplets are easily soluble in absolute alcohol and take the fat stains. This does not necessarily mean that the true interstitial granules contain no fatty substance nor that the fat droplets are composed wholly of fatty substances. Under normal physiological conditions the muscle fibers of both skeletal muscle and cardiac muscle of vertebrates may, and usually do, contain true interstitial granules as well as fat droplets.

I. MATERIAL AND METHODS

For the most part the material was obtained from the common laboratory animals: frog, mouse, rat, rabbit, cat, dog, pigeon, and from the bat. In a few cases the nutritive condition of the animal was altered by special feeding in the laboratory. Human material was used to a considerable extent and insect muscle was also examined.

The methods used in this study have a direct bearing on the chemistry of the granules and fat droplets, and will be discussed in some detail when considering that subject. The methods may be briefly outlined as follows:

1. Examination of fresh material.
 - a. Preparations made without the addition of fluids.
 - b. Preparations mounted in normal saline solution or 1 to 5 per cent solution of potassium hydroxide.
2. Tests of solubility of interstitial granules and fat droplets with alcohol, xylol, and with ether.
3. Examination of preparations stained by various methods.
 - a. Simple alcoholic solutions of Scharlach R, and Sudan III.
 - b. Herxheimer's Scharlach R.
 - c. Nile blue sulphate and Nile blue chlorhydrate.
 - d. Cresylviolett and Cresylechtviolett.
 - e. The methods of Weigert, Altman, Benda, and Regaud.

Herxheimer's ('01) stain is prepared by dissolving 2 grams of Na OH in 100 cc. of 70 per cent alcohol, Scharlach R then being added to saturation. The solution is filtered into a tightly closing vessel immediately before being used. Frozen sections or teased preparations of fresh tissue, or material used after two to twelve hours fixation in 20 per cent formalin, are washed in 60 per cent alcohol, transferred to the stain for three to fifteen minutes, washed in 60 to 70 per cent alcohol twenty to thirty seconds, followed by water, and mounted in levulose or glycerine. If alcohol washing is omitted precipitates are formed. Fat droplets are stained red, true interstitial granules and the protoplasm of muscle fibers are not colored. Alum-hematoxylin or Cresylechtviolett may be used as a counter-stain for the nuclei.

Nile blue: Teased preparations or frozen sections of fresh tissue or material used after a fixation of two to twelve hours in 20 per cent formalin, are stained fifteen minutes to two hours in a saturated aqueous solution of Nile blue chlorhydrate, washed in distilled water five minutes or more, and transferred to tap water. After five minutes in tap water the preparation should assume a reddish hue. If this does not occur a slight amount of alkali may be added to the water. The preparations are mounted in either levulose, potassium acetate, or glycerine. When Nile blue sulphate is used it is necessary to add a somewhat greater amount of alkali, to the tap water. Fat droplets are stained red; purple or blue, true interstitial granules are stained blue.

Cresylviolett: Fresh material, or material after two to twelve hours fixation in formalin, is stained in a dilute aqueous solution of Cresylviolett or Cresylechtviolett for ten to twenty minutes, then washed three to five minutes in distilled water and mounted in levulose syrup. Fat droplets are colorless, or (rarely) a faint red or blue, true interstitial granules are blue.

For the details of the complicated methods of Weigert, Altmann, Benda and Regaud, the reader is referred to *Encyklopädie der Mikroskopischen Technik*, Berlin, Wien, 1903, and to the discussion of Fauré-Fremiet, Mayer and Schaeffer ('10). Benda's method in particular is unnecessarily complex, requiring about two weeks to prepare a specimen, and its results are not uniform. Satisfactory preparations were obtained by a modified Weigert method which may be briefly stated as follows:

Fix twenty-four hours or more in a 10 to 20 per cent solution of formalin (4 to 8 per cent formaldehyde) with the addition of 0.75 per cent sodium chloride, then mordant in 5 per cent aqueous potassium bichromate four to seven days. Imbed in paraffin and section. Stain warm two to six hours in a mixture containing hematoxylin 1 gram and 2 per cent acetic acid 200 cc. Decolorize by use of Weigert's differentiating fluid, or by dilute (0.12 per cent) potassium permanganate followed by the oxalic acid-potassium sulphite mixture of the Pal-Weigert technique. Dehydrate, clear, and mount in balsam. Or prepare paraffin sections, as above, stain in Altmann's acid fuchsin, decolorize in picric

acid as used in Altmann's method and then clear and mount in balsam. The true interstitial granules are stained blue by hematoxylin or red if acid fuchsin is used.

II. NOMENCLATURE

In considering the significance of interstitial granules and fat droplets, a clear understanding of the terminology used by various authors is of importance. The true interstitial granules of Kölliker correspond to Altmann's granules or 'bioblasts,' to the 'mitochondria' of Benda, and to the 'plasmaosomes' of Arnold. Granules which do not correspond to the interstitial granules but are concerned in the formation of the myo-fibrills, are also included by Altmann, Benda and Arnold, as bioblasts, mitochondria and plasmaosomes. A part of the plasmaosomes of Arnold, those which are not colored by basic dyes, may represent fat droplets. Benda described mitochondria in fully developed smooth muscle, but thought that they do not occur in the sarcoplasm of adult skeletal muscle, while Holmgren and others, using the technique of Benda, have described such granules in skeletal muscle fibers. The 'exoplasmic granules' (J granules and Q granules) and the 'endoplasmic granules' of Holmgren correspond to the 'Sarcosomes' of Retzius which in turn correspond to Kölliker's true interstitial granules. It is possible that Retzius and Holmgren may have occasionally confused fat droplets with sarcosomes.

Albrecht ('02) classed the interstitial granules with his 'liposomes,' which could be demonstrated in all tissues by treating fresh preparations with 5 per cent potassium hydroxide. Since some of Albrecht's liposomes in striated muscle stained by acid fuchsin while others blackened with osmic acid, it is clear that he included as liposomes both the true interstitial granules and fat droplets. Albrecht must have been mistaken in thinking that all his liposomes are seen when fresh tissues are cleared in 5 per cent potassium hydroxide. Fat droplets are brought out clearly but the granules which stain with acid fuchsin are not apparent in such preparations although they may be seen when normal saline is used instead of the alkaline solution.

Bell ('10, '11) adopted Albrecht's term 'liposome.' As designated by him, none of the liposomes stain with acid fuchsin but all of them may be colored with Herxheimer's Scharlach R and all are soluble in alcohol. He describes certain of his liposomes as faintly-refractive and is evidently of the opinion that they correspond to the faintly-refractive interstitial granules of Knoll, Kölliker and other observers.

I find that the faintly-refractive granules described by Knoll ('80, '91) in the heart and skeletal muscles of the pigeon are readily stained by the acid fuchsin method but do not stain by Herxheimer's method. These granules would thus come within the category of liposomes as the term is used by Albrecht, but they form no part of the liposomes of Bell. The faintly-refractive liposomes of the latter author are faintly-refractive fat droplets and do not correspond to the faintly-refractive granules of Knoll. Knoll himself pointed out that his faintly-refractive granules correspond to the true interstitial granules of Kölliker.

The term true interstitial granules will be used in this paper to correspond to the true interstitial granules of Kölliker which as previously mentioned include a part of the bodies described by Altmann, Benda, and Arnold respectively, as bioblasts, mitochondria and plasmasomes.

The term 'fat' is here used to include lipoids and cholesterin compounds as well as the fatty acids and their glycerin esters.

III. RELATION OF INTERSTITIAL GRANULES AND FAT DROPLETS TO COLOR AND STRUCTURE OF MUSCLE

a. Light and dark muscle fibers

Krause ('64) found that the fibers of the red muscles of the rabbit contain more interstitial granules than are present in the fibers of white or pale muscles. Grützner ('84) described two types of fibers in human muscles, cloudy or dark and pale or white. He found that all human muscles contain both types of fibers and believed that dark fibers give macroscopically the red appearance to muscles while light fibers correspond to white or pale muscles.

Knoll ('89) concluded that the dark appearance of muscle fibers is due to the presence of interstitial granules, the dark fibers, he states, have many granules while light fibers are free from granules or contain only a small number. Knoll ('91) described the dark fibers as rich in interfibrillar substance or sarcoplasm ('protoplasma reich') and as containing many interstitial granules, while the light fibers were poor in interfibrillar substance (protoplasma arm') and contained few granules. As a rule, red muscles contained more dark fibers while white muscles consisted of light fibers to a large extent. The dark fibers were usually of a smaller diameter than light fibers. Knoll also held that in general the active muscles contain a larger proportion of dark muscle fibers with a corresponding increase in the number and size of the interstitial granules. Schaeffer ('93) confirmed the work of Grützner and Knoll. He also found that dark fibers showing fixed contraction nodes simulate light fibers. Bell ('11) found that, in general, dark fibers contained coarse, strongly-refractive 'liposomes,' while light fibers contained small faintly-refractive liposomes. He stained the liposomes (fat droplets) with Herxheimer's solution of Scharlach R and in this way demonstrated the types of fibers.

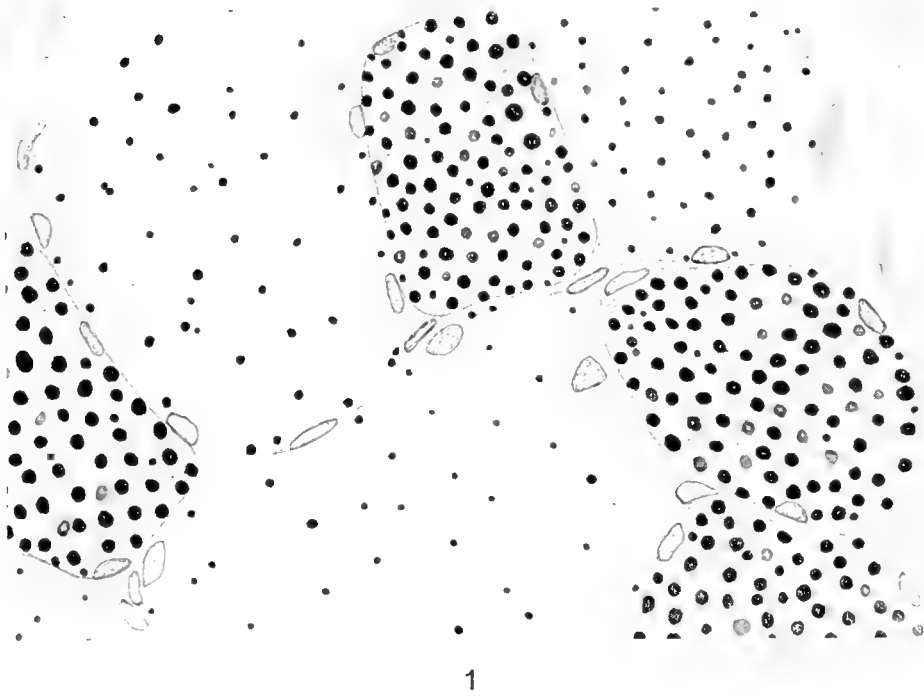
The reader is referred to the papers of Knoll, Schaeffer, and Bell for an account of the general occurrence and distribution of light and dark fibers in different animals. The work of these authors has shown that differences in opacity, corresponding to light, dark, and intermediate fibers, occur in the striated muscles of many animals, including practically all those most commonly employed in the laboratory.

In my studies the light and dark fibers were easily observed in transverse sections of fresh tissue cut on the freezing microtome. The difference in opacity is most marked when the specimen is mounted in normal saline and examined with low magnification, the reflected light being cut off by a paper shield or by some other means. The types of fibers were also demonstrated by staining frozen sections with Herxheimer's Scharlach R, with Nile blue, or with Cresylviolett. In the pectoral muscles of the pigeon

where the true interstitial granules are large and numerous, the types of fibers were distinguished by the Weigert and Altmann methods as well as by the other methods just mentioned.

The light, dark and intermediate types of muscle fibers are clearly marked in unstained frozen sections of fresh material from normal cats, dogs and rats. If such sections be placed in absolute alcohol for a few minutes and subsequently examined under the microscope the dark and intermediate fibers assume the appearance of light fibers. The fat droplets and also the alcohol-soluble portion of the true interstitial granules have been removed by the alcohol, and the removal of these droplets and granules causes the dark fibers to lose their opacity. In the dog, cat and rat the true interstitial granules are small and the opacity of the dark fibers is largely due to fat droplets. In the pectoral muscles of the pigeon and the bat, not only fat droplets but also true interstitial granules are an important factor in causing the dark appearance of fibers. Sections of formalin fixed material from the pectoral muscles of the pigeon after treatment with absolute alcohol ten to twelve hours still show the dark fibers much more opaque than the light, while in similar preparations from the dog, cat or rat, dark fibers have lost their opacity and have the appearance of light fibers. The fat droplets have been removed by the alcohol but the large true interstitial granules of the pectoral muscles of the pigeon remain and cause the opaque appearance of the dark fibers to be retained. The staining reactions and solubility of fat droplets and true interstitial granules will be dealt with later, but I may here mention that formalin coagulates the true interstitial granules in such a manner as to partially protect them from the action of fat solvents while fat droplets are readily soluble both before and after formalin fixation.

Figure 1 represents the types of fibers in the pectoralis major of an exceptionally well nourished white rat. The preparation was stained by Herxheimer's method. Figure 2 shows a similar specimen from a very emaciated rat. The dark fibers so apparent in the well nourished animal have largely disappeared in the poorly nourished animal. The granules shown in the figures are



1



2

Fig. 1 Transverse section of muscle fibers from the pectoralis major of a rat which had been fed on fat meat. Fat droplets (black) are stained red by Herxheimer's Scharlach R. The larger fibers are 'light fibers', the 'dark fibers' are smaller. $\times 600$.

Fig. 2 Transverse section of muscle fibers from the pectoralis major of an emaciated rat. Fat droplets (black) are stained red by Herxheimer's Scharlach R. Both 'light and 'dark' fibers are shown. $\times 600$.

fat droplets. Bell ('11) first clearly demonstrated that the fat content of muscle is largely dependent upon the nutritive condition of the animal. This subject will be referred to later.

The relative number of light, dark and intermediate fibers is known to be exceedingly variable. For any given muscle of a given species the percentage of each type is fairly constant under normal nutritive conditions, although individual variation occurs. Dark fibers have more interfibrillar substance or sarcoplasm and are commonly of lesser diameter than light fibers. However, the dark fibers in eye muscles (human) are as large or even larger than the light fibers. In the pectoralis major of the pigeon light fibers are exceptionally large with nuclei placed in the substance of the fiber, while dark fibers are small and the nuclei are peripherally situated. Mammalian skeletal muscle, in so far as I have observed, has peripherally situated nuclei in both types of fibers. The pectoral muscles of the bat are peculiar in that the fibers are all small and correspond to dark fibers as found in the pigeon. The fat content of the dark fibers of the bat as shown by Herxheimer's Scharlach R varies somewhat in the different fibers and this may be considered as an indication of the two types.

The two types of fibers, dark and light, are clearly marked in the human fetus of seven months and of eight months. I have also found the two types in the ox fetus from 45 to 65 cm. and at full term. In so far as I know dark fibers in the fetus have not been previously described.

b. Relation of light and dark muscle fibers to white and red muscle

Red muscles, as the pectoralis major of the pigeon and bat, commonly show a high percentage of dark fibers, while the white muscles of the rabbit and certain other animals may be made up largely or wholly of light fibers. By many text books and by even a recent author, Ewald ('10), the terms red and white muscle fibers are used synonymously with dark and light fibers. Such a terminology is probably founded upon a misconception and should be discontinued. The white muscles of the frog during the winter season show under the microscope a large percentage of very dark fibers and frequently red muscles, as cardiac muscle

in certain individuals, show only light fibers. It is clear that dark fibers do not necessarily give a red color to muscle nor does the presence of light fibers in red muscle make it less red in appearance.

According to Krause ('11) red and white muscles in the rabbit differ only in number and arrangement of blood vessels and in amount of connective tissue. I am unable to say to what extent the red color of muscle is due to the presence of blood. Lelièvre and Retterer ('09) studied the structural differences between the red and white muscles of the rabbit. They concluded, among other things, that the membrane of Krause (Z, Strie d'Amici) is absent from white muscle (adductor magnus). I have examined the fibers of the adductor magnus of the rabbit and find the membrane of Krause present and clearly visible in both fresh and fixed preparations.

c. Morphology and position of fat droplets

Krause ('73) observed a regular arrangement of the interstitial granules in transverse rows situated in segment *J* on either side of Krause's membrane, *Z*. The observations of Krause have been confirmed by Retzius ('90), Arnold ('00), Holmgren ('07,-'10), and many others, and it has also been observed that the position of the granules as described by Krause applies to that of both the true interstitial granules and fat droplets. Retzius, Holmgren and others have described large interstitial granules as occurring in the anisotropic segment *Q*, (Brücke's disc).

The fat droplets of muscle fibers are in general spherical. Their form may be modified by the pressure of the muscle columns. Droplets that have a diameter exceeding one micron frequently show a certain amount of elongation in the direction of the longitudinal axis of the fiber. Exceptionally, the longitudinal diameter of elongated droplets is nearly twice the transverse diameter. Upon contraction of the fiber, droplets become more spherical or even flattened in the transverse direction. Occasionally, and especially in human muscle, the fat has a granular form, the evenly rounded contour of droplets being absent. Possibly this is due to post mortem changes. I am not here referring to the pigment

granules of irregular form so frequently present in human muscle, especially in cardiac muscle.

Fat droplets occur in varying sizes. Apparently the smallest droplets are beyond the limit of microscopic vision. Bell ('11), staining muscle fibers by Herxheimer's method, shows 'liposomes' (his fig. 4, plate 16) which measure less than 0.5 μ . after a magnification of 1300. This means that they have a diameter of less than 0.5 μ . According to Heidenhain ('00), 0.2 μ is the extreme lower limit of microscopical vision (n. a. l. 4), and if perchance any structure of smaller size were visible it would still appear to have a diameter of 0.2 micron. Herxheimer's Scharlach R, as well as Nile blue frequently forms precipitates in the tissues but precipitate granules lack the refractive character of fat droplets and when of appreciable size the two are easily distinguishable. Precipitate in a finely granular form may be confused with minute fat droplets, and for this reason I have preferred to regard as probably a precipitate, all granules having an approximate diameter of 0.5 μ or less. The diameter of the fat droplets, which differs with the nutritive condition and with the species of the animal, seldom, if ever, exceeds 3 μ in normal mammalian muscle. Fresh preparations stained in Herxheimer's Scharlach R often show droplets as large as 5 to 6 μ , but the examination of fresh material to which no foreign fluids have had access has convinced me that such large globules arise by the confluence of smaller droplets.

The fat droplets seldom show a regular arrangement within muscle fibers in preparations made from fresh material without fixation. To demonstrate the position of fat droplets, portions of muscle were stretched upon card board or small pieces of glass and while still warm placed in 20 per cent formalin in a 0.75 per cent solution of sodium chloride. After fixation of two to twenty-four hours, sections were cut on the freezing microtome and stained by Herxheimer's method or by the Nile blue method.

Figure 3 shows the droplets in a portion of a longitudinal section of a dark muscle fiber from the pectoralis major of an adult cat. The droplets are in transverse rows in segment *J* on either side of the membrane of Krause, *Z*. An arrangement in longitudinal rows is also apparent. Figure 4 represents a portion of a light

fiber from the same specimen. The droplets are fewer in number and somewhat smaller than in the dark fibers. The arrangement is similar in the two types. Droplets may be smaller than those shown in the figures and placed nearer to the membrane *Z*, thus making it difficult to see two distinct rows. Droplets in dark fibers which show a large quantity of fat, may all be of small size, not exceeding 1μ and arranged as in figure 3. Dark fibers may show longitudinal rows of small droplets placed at frequent intervals between less frequent rows of larger, elongated droplets.

In transverse sections the droplets are situated between the muscle columns or Cohnheim's areas. The larger droplets are at nodal points in the sarcoplasmic net work. No droplets are found between the individual myofibrils within the muscle columns. In muscle fibers of a type which have large true interstitial granules (granules of segment *Q*, pectoral muscles of the pigeon and bat and in cardiac muscle), the fat droplets when small are placed in segment *J*, while larger droplets extend into segment *Q*. In this type of muscle the droplets in segment *J* may be placed on either side of the membrane of Krause, *Z*, but frequently droplets are in a single row occupying the position of the membrane. Fat droplets are also of almost constant occurrence in the sarcoplasm beneath the sarcolemma and surrounding the muscle fiber nuclei of skeletal muscle, and they are equally constant in the central perinuclear sarcoplasmic accumulations of cardiac muscle.

d. Morphology and position of true interstitial granules

Figure 5 represents the true granules in one of the dark muscle fibers of the pectoralis major of the pigeon, longitudinal section, Weigert method. The granules are rod shaped, being approximately 1μ in diameter and 2μ in length. In position they correspond to segment *Q* and represent Holmgren's *Q* granules. Figure 6 represents a portion of a similar fiber, from the same muscle, stained with Nile blue sulphate after formalin fixation. By this method true interstitial granules are stained blue while fat droplets are colored red. The fat droplets, for the most part, are situated in segment *J* at the poles of the true interstitial gran-

ules. Occasionally the substance of the *Q* granules appears to partially surround a fat droplet. In formalin-bichromate material with the Weigert and Altmann methods, vacuoles left by the extraction of fat droplets during the paraffin process, are sometimes seen within the substance of the granule but usually the vacuoles are in segment *J* at the poles of the granules as shown in figure 5. The vacuoles thus correspond in position to the polar fat granules which Holmgren describes as of occasional occurrence.

Transverse sections show the true interstitial granules between the muscle columns. When demonstrated by the formalin-bichromate Weigert or Altmann methods, they appear rounded; or flattened in transverse section. In frozen sections stained with Cresylviolett or Nile blue sulphate, the granules are seen as stellate bodies which may occupy almost the entire space between the muscle columns (fig. 7). In describing this appearance in unstained preparations, Kölliker ('88) speaks of granules provided with wing shaped processes.

When frozen sections of formalin fixed material are treated with absolute alcohol and subsequently stained, Cresylviolett stains the true interstitial granules (pectoral muscles of the pigeon) rather faintly but the processes which give the stellate or irregular

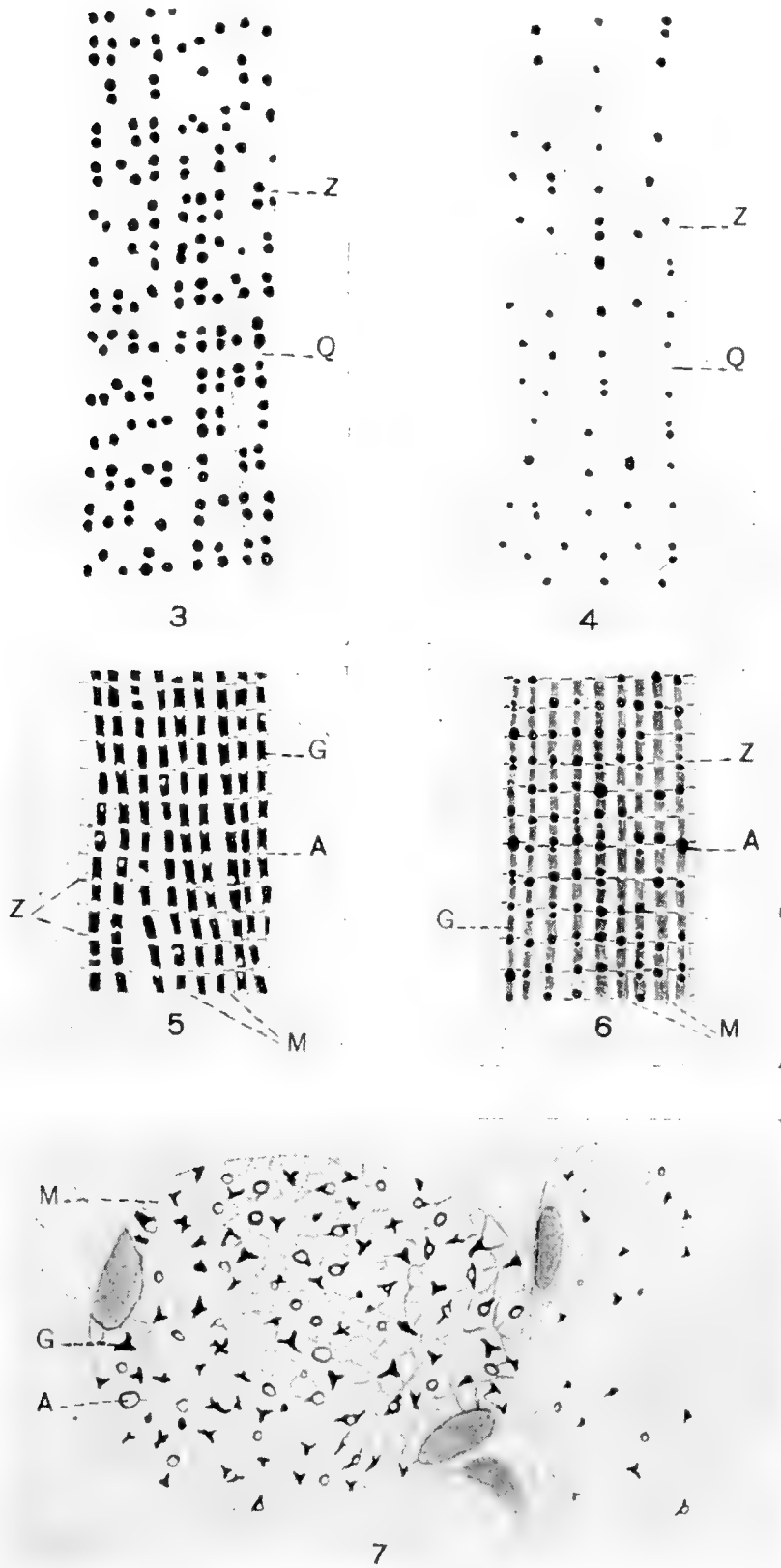
Fig. 3 Portion of a longitudinal section of a dark muscle fiber from the normal pectoralis major of an adult cat. Fat droplets (black) are stained red by Herxheimer's Scharlach R. Membrane of Krause is situated at *Z*; *Q*, position of anisotropic disc. $\times 1500$.

Fig. 4 Portion of a longitudinal section of a light muscle fiber from the normal pectoralis major of an adult cat, stained as in figure 3; *Z*, position of Krause's membrane; *Q*, position of anisotropic disc. $\times 1500$.

Fig. 5 Portion of a longitudinal section of a dark muscle fiber from the normal pectoralis major of a pigeon. True interstitial granules, *g*, (black) are stained blue by a modified Weigert process. Fat droplets appear as vacuoles, *a*. The letter *m* indicates muscle columns; *z*, Krause's membrane. $\times 1500$.

Fig. 6 Portion of a dark muscle fiber from the normal pectoralis major of a pigeon, stained by Nile blue. Fat droplets, *a*, are stained red (black); true interstitial granules, *g*, are stained blue (gray); *m*, muscle columns; *z*, Krause's membrane. $\times 1500$.

Fig. 7 Transverse section of a dark fiber and a portion of a light fiber from the normal pectoralis major of a white rat, stained with Cresylviolett. Fat droplets, *a*, are colorless or a faint red, true interstitial granules, *g*, are stained blue (black). The letter *m* indicates muscle columns. $\times 1500$.



appearance in transverse section are evidently not present. Nile blue still stains the granules with intensity but no stellate forms are visible. In this process, the fat droplets have been removed by the alcohol and also much of the substance which stains with Cresylviolett has apparently been extracted from the fiber or rendered incolorable. In formalin-bichromate material stained as either Weigert or Altmann preparations, paraffin sections, the granules seldom present the stellate form. The wing shaped processes of Kölliker may be dissolved during the process of paraffin embedding. Holmgren, however, has sometimes demonstrated the wing shaped processes by Benda's mitochondrial method.

The true granules were described above as having a rod shaped form in the breast muscle of the pigeon. This is not to be taken as invariably true. I have also observed in the pigeon muscle, dumb-bell and diplosomic forms in longitudinally cut fibers. At other times the shape is irregular and the granule is not wholly confined to the anisotropic segment. The longitudinal rows of granules in the light fibers of the pectoralis major of the pigeon are placed at greater intervals than in dark fibers though the granules are somewhat smaller in the former.

The true granules of the pectoral muscles of the bat are similar in number, size, and position to those of the pigeon. However, in the skeletal muscle of most mammals (dog, cat, rabbit) the granules are of smaller size and fewer in number. In longitudinal sections they may be dumb-bell shaped, rodules, or slender thread structures, either confined to segment *Q* or extending through the entire distance between adjacent membranes of Krause, *Z*. They may occur as spherical bodies having a diameter of 1μ or less and situated in segment *J* on either side of Krause's membrane. The appearance is then similar to that shown for fat droplets in figure 3. The occurrence of true granules in segment *J* is described by Holmgren ('10) as typical for mammalian skeletal muscle. The stellate or irregular forms in transverse sections shown by staining with Cresylviolett were of constant occurrence in all the animals used in this investigation. They may be demonstrated in both light and dark fibers, even when granules appear to be absent by the Weigert and Altmann methods.

In cardiac muscle (pigeon, dog, rat) the true interstitial granules are present in very striking numbers. They are usually confined to the segment *Q* and correspond to Holmgren's *Q* granules. Regaud ('09) described and figured the granules in the cardiac muscle of the dog as plate-like structures, confined to segment *Q* and extending radially between the muscle columns from the periphery of the fiber toward the central sarcoplasmic column. I have examined the cardiac muscle of several dogs and find the true interstitial granules as described by Regaud.

In the wing muscle fibers of insects the granules are very similar in form and position to either the granules of the pectoral muscle the pigeon and the bat or to those of cardiac muscle in vertebrates. The wing muscle fibers of the *Belostoma Americana* show rounded granules in transverse section similar to those of the pigeon. The fibers of dragon flies have granules of a plate-like form similar to those of the cardiac muscle of the dog. I have not examined fibers from the leg muscles of insects.

Retzius ('09) believed that the interstitial granules are united and held in position by a fibrous network, which he demonstrated with gold chloride. A comparison of gold chloride preparations with others made by the various methods already mentioned, leads me to believe that the appearance of a net work uniting the interstitial granules is to be interpreted as a precipitate of gold.

The position assumed by the granules appears to be determined solely by their size and the pressure of the muscle columns. Perhaps also the position of both the true interstitial granules and fat droplets may be taken as affording evidence in support of the now commonly accepted view that the membranes of Krause are present in the sarcoplasm between the muscle columns. The dumb-bell and diplosomic granules may be formed by a thickening of the muscle columns at Hensen's line. After fixation, and due to the process required for embedding, the substance of the column shrinks leaving its impress upon the granules. The plate-like forms occur in types of muscle that present a radial arrangement of the muscle columns. The substance of the granule occupies the space between the columns and thus, in transverse sections, appears in the form of a plate.

In general it may be said that the shape of the true interstitial granules indicates that in fresh, unfixed muscle they are composed of a plastic, yielding substance which easily takes the form imposed by surrounding structures of a more resistant nature. Probably the term granule is a misnomer, but it is here used because it has become firmly fixed in the literature.

Kölliker ('88), Holmgren ('07, '10), Thulin ('09) and Knoche ('09) believed that the true interstitial granules possess a limiting membrane. In Weigert, Altmann, Cresylviolett and Nile blue preparations, I have observed nothing which can be taken as indicating the existence of such a membrane. The membrane-like appearance in fresh unstained preparations is, in all probability, an optical effect due to differences in refractive index.

IV. GENERAL OCCURRENCE OF INTERSTITIAL GRANULES AND FAT DROPLETS

General occurrence of true interstitial granules

Kölliker ('89) described the true interstitial granules as of constant occurrence, sometimes in enormous numbers, in the striated muscle fibers of all classes of vertebrates and insects. Knoll ('91) observed the true interstitial granules in a large number of animals including amphibians, reptiles, birds and mammals. Retzius ('09) described his sarcosomes (true interstitial granules) in insects and mammals. Altmann ('94) demonstrated the granules in insects and in the frog. Holmgren ('07-'10) described Kölliker's granules as occurring in insects and in vertebrates, rabbit, guinea pig, rat and white mouse.

The large true interstitial granules are included in nearly every description of insect muscle. Similar granules in vertebrate muscle, although described by the investigators just mentioned and by many others, have frequently been overlooked. This applies not only to text books but likewise to recent original articles.

The white muscles of the rabbit show, by the Weigert or Altmann method, only a few granules or none at all and the red muscles may show but a limited number. In the dark fibers of the dog and the gray rat, granules are larger and somewhat more

numerous than in the rabbit, especially in the muscles of the tongue and in the diaphragm. In the powerful and active pectoralis major of the pigeon and of the bat, the true interstitial granules as demonstrated by the Weigert method are relatively large and occur in great numbers in each fiber (fig. 5). In so far as I have observed, the granules which stain blue with Cresyl-violett, (fig. 7), may be demonstrated in considerable numbers in every striated muscle fiber of all vertebrates. As already mentioned, granules to be demonstrated by this method do not always correspond with those shown by the Weigert and Altmann methods.

In cardiac muscle, as pointed out by Kölliker, Knoll and Holmgren; the granules are especially abundant. I have examined the heart muscle of the dog, the rat and the pigeon. The number and size of the granules is very striking. I have not been able to demonstrate the true interstitial granules in human cardiac or skeletal muscle, due doubtless to the fact that fresh material was not obtainable.

General occurrence of fat droplets

Kölliker ('88, '89) describes fat droplets as of general occurrence in muscle fibers of insects and vertebrates. However, he was evidently somewhat in doubt as to their being true fat droplets. He speaks of the droplets as fat like granules or as the long known dark (fat?) granules. Walbaum ('99) examined the muscles of 119 human bodies. He found fat droplets in some of the fibers of about two-thirds of the cases examined. Droplets were most numerous in the eye muscles and of very infrequent occurrence in the diaphragm. Ten per cent formalin was used as a fixative. He examined teased preparations in water and in normal saline and observed that many of the fatty droplets are left unstained by Sudan III. Retzius ('91) believed that fat droplets are not normally present in muscle fibers. Among others Stadkewitch ('94), Ricker and Ellenbeck ('99) and Kemp and Hall ('07), may be mentioned as failing to find fat droplets in the normal muscle fibers of adult vertebrates. As is well known, the muscle fibers of the winter frog are crowded with fat droplets while such drop-

lets are usually supposed to be absent in summer frogs caught in the field.

Fat droplets have often been overlooked in skeletal muscle due to the fact that they are so frequently lost in the fixatives employed (formalin) and may often be left unstained by osmic acid and Sudan III. Bell ('11) who employed Herxheimer's Scharlach R on fresh tissue has demonstrated that 'liposomes' occur under normal conditions in all vertebrate muscle. He states that the number and size of the liposomes vary in different species and individuals and also with nutritive condition. He examined no human muscle.

The dog, cat (figs. 2 and 3) and rat (figs. 1 and 2) may be mentioned as examples of animals commonly having a large quantity of fat in their skeletal muscle fibers while the fibers of the ox and the rabbit have considerably less. I think that an extensive investigation might show that fibers of herbivorous animals do not store fat to such a great extent as is the case in carnivora.

Human skeletal muscle. I have examined some of the muscles, usually diaphragm, pectoral and eye muscles from about twenty-five autopsies and conclude that fat droplets occur constantly and abundantly in normal human muscle. Fat in the diaphragm was present in large amount in about half the cases examined. In two or three cases the droplets in the fibers of this muscle were few in number or possibly absent but I think that this may be attributed to pathological conditions, poor nutrition or to post mortem change. I have never failed to find fat in human eye muscle, usually in large amount.

Cardiac muscle. I have examined sections from the right ventricle of the hearts of about twenty-five dogs and cats, a dozen rats and several mice. Fat in varying amounts was found within the muscle fibers of all these animals. In two dogs only a few small droplets were to be seen, but usually in this animal fat was present in moderate amount. An exceptionally large amount of fat was present in the cardiac muscle fibers of a well nourished rat and of a pregnant cat.

Human cardiac muscle. Of fifteen hearts, fat droplets were present in ten. Two or three of the remaining five were examined

after most decided post mortem changes had taken place. Fat droplets in the human cardiac fibers are commonly regarded as occurring only under pathological conditions. I believe that a thorough investigation of the subject, with the aid of the best technique would demonstrate that fat droplets of small size are of normal occurrence in human cardiac muscle fibers.

Fetal muscle. Kainath ('04) examined the skeletal muscles of the ox fetus. He found fine fat droplets in the fibers from the 3.5 to the 12.5 cm. stage but none were present in the fetus of 20 cm. and 40 cm. Bell ('09) found fat in the muscle fibers of the ox fetus from the 7 to the 28 cm. stage, but observed none in the fibers of seven fetuses of later stages.

The muscle fibers of the early fetus were not examined in this study but I have found a large amount of fat in the fibers of the ox fetus from the 35 cm. stage to full term. The dark fibers contain many fat droplets while light fibers have but a small number. I have also examined the skeletal muscles of a seven months and eight months human fetus. The dark fibers were crowded with fat droplets.

V. CHEMICAL NATURE OF INTERSTITIAL GRANULES AND FAT DROPLETS

A qualitative chemical analysis, in vitro, of the true interstitial granules and fat droplets of muscle fibers is beset with obvious difficulties and such an investigation has never been attempted. One can, however, draw certain conclusions respecting the chemistry of these bodies by a consideration of the nature of the various methods used in demonstrating them in tissue sections.

a. Chemical nature of true interstitial granules

Kölliker ('57) states that the true interstitial granules, being very pale, especially in mammalian muscle, have been overlooked by previous observers. He finds the granules insoluble in alcohol and ether. Kölliker ('88) concludes that, chemically, the granules are identical with no known substance. They contain no glycogen for they do not give the iodine reaction. Retzius ('90)

considers the true interstitial granules to be of a non-fatty nature. He terms them 'sarcosomes' in order to distinguish them from pathological fat droplets. Knoll ('80, '81, '91) believes the true interstitial granules of Kölliker to have a fatty marginal layer and a central portion possibly of lecithin. Arnold ('07) thinks that the glycogen of striated muscle is bound to the sarcosomes. He observed that sarcosomes which contain glycogen stain by Best's carmine method ('06) while those that are free from glycogen remain colorless. Regaud and Favre ('09) demonstrated granules in the tongue muscles of the rabbit by Regaud's formalin-bichromate iron-hematoxylin method. They believed these granules to correspond to Kölliker's granules. Chemically they were thought to be an albumino-lipoid. Bell('11) finds that the large *Q* granules of insects contain no fatty substance and are widely different chemically from the interstitial granules of vertebrate muscle. He thinks that the microsomes of Altmann may be artefacts, and is evidently of the opinion that other observers have mistaken fat droplets in vertebrate muscle for the true interstitial granules.

1. *Refractive character.* Fibers from the pectoralis major of the pigeon or the wing muscles of an insect may be teased and placed, without the addition of fluid, upon a slide, the cover glass being applied with slight pressure. Such preparations show the fat droplets as highly-refractive globules but the true interstitial granules seem to have approximately the same refractive index as the substance of the muscle columns and are not clearly visible. However they may be seen as faintly-refractive bodies after normal saline has been drawn under the cover-glass.

2. *Solubility.* As has been observed by Kölliker and others the true interstitial granules are disintegrated and partially dissolved by water. In order to test the effect of fat solvents upon the granules I have examined sections prepared by the paraffin process after fixation in 97 per cent alcohol. In sections from the heart or pectoral muscles of the pigeon, the granules in alcohol fixed material are seen as broken fragments. A comparison of these sections with others made after formalin-bichromate fixation shows that a large part of the substance of the granules has disappeared from the alcohol fixed material. This suggests the idea

that the partial disappearance of the granules from alcohol fixed material may be due to the solution of a fatty substance which can be rendered insoluble by the action of potassium bichromate. It was found, however, that the granules of material fixed in 20 per cent formalin show no more shrinking after the paraffin process than do those of formalin fixed material which has been mordanted in potassium bichromate before the alcohol and xylol preceding embedding. Thin paraffin sections were also washed in several changes of hot ether four to six hours and subsequently examined under the microscope after staining with acid fuchsin or hematoxylin. The fat extraction by this method is considered more complete than by the Soxhlet's apparatus as ordinarily employed. Ether does not dissolve the true interstitial granules from paraffin sections of formalin fixed material taken from the pectoral muscles of the pigeon. The partial disappearance of the granules, from alcohol fixed material, takes place in the alcohol and xylol, and the subsequent treatment with ether appears to have little effect. If we suppose that the shrinkage or disappearance of the granules in alcohol or xylol is due to the extraction of a fatty substance, it is also necessary to suppose that the fatty substance is in part rendered insoluble in alcohol, xylol and ether by the coagulative action of the formalin on the non-fatty substance of the granules. As will be seen below, however, staining with Cresylviolett indicates that the true interstitial granules are soluble in alcohol to a very considerable extent even in formalin fixed material

3. *Results with Cresylviolett R R, Cresylechtviolett, and Nile blue sulphate.* Krause ('11) recommends Cresylviolett R B in dilute aqueous solution for demonstrating the interstitial granules in fresh tissue. I have used Cresylechtviolett and Cresylviolett R R which are apparently similar to the dye employed by Krause. The fat droplets are not stained to any considerable extent by Cresylviolett. Occasionally they show an exceedingly faint red color or a more intense peripheral blue staining but usually they are left colorless.

As mentioned above, the true interstitial granules are partially dissolved by water. Since this is the case, one should

not expect to obtain a true picture of the granules by the use of an aqueous staining solution on unfixed material. The arrangement of the granules is often very irregular in Cresylviolett preparations of unfixed material especially if the section is exposed to the action of water previous to staining or left too long in the stain, or if the material is taken from animals following rigor mortis. Under such circumstances granules are absent from portions of the fiber and are aggregated in masses within other portions of the fiber or beneath the sarcolemma. The blue stained substance, however, is not easily, if at all, soluble in water. Aqueous solutions of Cresylviolett may also be applied to frozen sections of material fixed fresh for two to twenty-four hours in 20 per cent formalin in a 0.75 per cent sodium chloride solution. Such preparations show a comparatively uniform arrangement of the blue staining granules corresponding to that of the true interstitial granules of Kölliker.

It has already been mentioned that the wing shaped processes of Kölliker, which give the granules a stellate appearance, are not stained by Cresylviolett when the section has been previously treated with alcohol. In formalin fixed material the large granules of the pectoral muscles of the pigeon can be stained intensely with Nile blue or faintly with Cresylviolett even after the action of alcohol. The true interstitial granules in the muscle fibers of the dog, cat, rat and rabbit stain with Cresylviolett and for the most part appear to be soluble in alcohol both in fresh material and in formalin fixed material for they cannot be stained when sections have been previously treated with absolute alcohol. When this material has been kept in 20 per cent formalin for several weeks, the wing-shaped processes and soluble granules seem to have disappeared and can no longer be demonstrated by Cresylviolett or Nile blue sulphate. Even after prolonged exposure to formalin the true interstitial granules in the pectoral muscles of the pigeon are still readily stained by Nile blue sulphate and faintly colored by Cresylviolett.

If it be supposed that the alcohol-soluble substance of the true interstitial granules is a form of fat, the fact that it stains with basic dyes may indicate that it is a lipid or fatty acid.

4. *Results with the methods of Weigert, Altmann, Benda and Regaud.* To demonstrate the true interstitial granules, Altmann ('94) employed his bichromate-osmic acid-fuchsin method. Holmgren ('10) made use of Bend's mitochondrial method and Regaud ('09) used his formalin-bichromate iron-hematoxylin method. I find that the granules may be demonstrated in a satisfactory manner by any of the above methods as well as by the Weigert method which involves formalin-bichromate fixation followed by hematoxylin staining. Similar results by these methods is to be expected for the methods are chemically similar although the stains employed, acid fuchsin, Crystallviolet, hematoxylin, and iron-hematoxylin, are of a varied character.

Smith, Mair and Thorp ('08) have explained the chemistry of the Weigert hematoxylin process. They found that the method depends upon the oxidizing action of potassium bichromate upon unsaturated fats. The oxide of chromium forms with the fat molecules a compound which is insoluble in fat solvents and capable of forming a lake with hematoxylin. It is only during the process of oxidation that the fat-chrome compound forms the hematoxylin lake. After complete oxidation the staining no longer takes place. These observers found the method applicable not only to unsaturated fats, as oleic acid and triolein, but also to lipoids in which unsaturated groupings occur such as cholesterin and cerebrosides. The work of Smith, Mair, and Thorp was confirmed and extended by Fauré-Fremiet, Mayer, and Schaeffer ('10). They found that not only the unsaturated but also certain of the saturated fatty acids, including palmitic, are rendered insoluble in alcohol and xylol by oxidizing reagents and also by the action of salts of the heavy metals. (Benda explained the action of the salts of copper on fatty acids as depending upon the formation of insoluble copper soaps.) These insolubilized fats were stained with more or less intensity by both acid and basic anilin dyes and in certain cases (after copper or chromic compounds, salts of iron and of zinc) a hematoxylin lake was formed. The phosphatid lipoids were not rendered insoluble in xylol by the action of salts of the heavy metals, but were insoluble after chromic and certain other

oxidizing compounds. Lipoids rendered insoluble by chromic compounds stained with considerable intensity by Orange G but could be stained with the anilin dyes only when the potassium bichromate had been kept warm during the process of oxidation. The hematoxylin lake in the case of the lipoids, did not follow excepting after a mordant such as iron alum. It was also observed that both albumino-lipoids (lecithalbumin) and mixtures of fatty acid and albuminoids were precipitated by formalin in such a way as to render the fatty substances practically insoluble in ordinary fat solvents. For example, oleic acid in a precipitated albuminous mixture was stained by various methods, even after the action of alcohol and alcohol-ether for several days at a temperature of 35°C. The methods of Altmann, Benda and Weigert, although variously modified are, according to these observers, based on the same chemical principles and give almost identical results when applied to the mitochondria (Altmann's granules or the true interstitial granules of this paper). After an extended inquiry into the chemistry of the mitochondria, they conclude that the granules contain a fatty body which is neither a neutral fat nor a soap but is probably an unsaturated fatty acid, absorbed by an albuminous granule or present in an albumino-lipoid compound.

If we assume with the authors just quoted and with Regaud and Favre ('09) that the true interstitial granules are an albumino-lipoid or a fatty-acid albuminous mixture, the action of formalin in partially protecting them against fat solvents is explained in that the albuminous component is coagulated by the formalin and the fatty component is thus rendered less easily extractable. The same assumption also permits us to explain the action upon the granules of the methods of Altmann, Benda, Weigert and Regaud. It would, of course, be a mistake to consider these methods as specific for albumino-lipoids. They are of wide application and do not afford distinctive evidence as to the chemical nature of the substances stained.

5. *Results with acid fuchsin.* Knoche ('09) obtained a micro-chemical xanthoproteic reaction with the true interstitial granules of Kölliker and believed that the proteid thus shown was an

albuminous substance. He states that the granules have a capsule which stains with acid fuchsin but he does not give the details of his technique. As has been previously mentioned, the entire granule is readily stained in formalin-bichromate material with Altmann's acid fuchsin. Employing material from the pectoral muscles of the pigeon and bat and from the wing muscles of insects, I have also found that Altmann's acid fuchsin stains the granules after simple formalin saline fixation (paraffin process) the potassium bichromate being unnecessary. The fragmented granules in alcohol-fixed material are not stained by Altmann's acid fuchsin.

Smith and Mair ('11) find that lecithin and sphingosine stain readily with acid fuchsin both before and after the action of potassium bichromate. They think the presence of either of these substances would explain the staining of Altmann's acid fuchsin granules. Pure lecithin, according to these observers, does not stain by the Weigert process, but they add that it stains readily if it has the slightest admixture of cholesterin. Fauré-Fremiet, Mayer and Schaeffer ('10) state that lecithin and other lipoids fail to stain by the Weigert process, but may be stained by hematoxylin if preceded by iron alum. Since the true interstitial granules stain readily by the Weigert process, the iron alum not being necessary, we may conclude that if the staining of these granules depends largely on lecithin, as suggested by the acid fuchsin method, the lecithin is not in a pure state.

Fatty acids, according to Smith and Mair ('11), do not stain with acid fuchsin either before or after the action of potassium bichromate. Fauré-Fremiet, Mayer and Schaeffer ('10), on the other hand, find that fatty acids are faintly stained by this dye, both before and after bichromating, presumably with greater intensity in the latter case, for they think that the presence of fatty acid would account for the staining of Altmann's granules. I have stained tissue paper smears of oleic acid (Kahlbaum) after treatment for a variable length of time (one to six days) in saturated potassium bichromate. The smears were stained with acid fuchsin either Altmann's mixture or in alcoholic solution, heating according to the method of Altmann. The droplets were stained

a somewhat pale red. The color in the case of oleic acid appears to be too faint to fully account for the intense red of the true interstitial granules, and thus it is doubtful that these granules contain a pure oleic acid.

6. *Results with Sudan III, osmium tetroxide, gold chloride.* Sudan III does not color the true interstitial granules unless we take into account an extremely faint yellow to be obtained after the action of potassium bichromate. The granules are slightly darkened, but not blackened, by 2 per cent osmic acid followed by pyroligneous acid or by alcohol for reducing the osmium. Retzius ('90), Knoll ('91), and others have stained the true interstitial granules with gold chloride but the gold precipitate is not considered differential for the presence or absence of fat.

7. *Summary.* The observations presented above are of too general a character to permit of definite conclusions as to the chemical nature of the true interstitial granules of Kölliker. It is certain that the granules contain a non-fatty element, probably of a proteid nature. It may be stated that the substance upon which depends their staining by basic dyes, as well as by the more complex methods of Altmann, Weigert and Regaud, is a substance soluble in fat solvents. In part this soluble substance may be protected from fat solvents by the action of formalin as well as by chrom-osmic mixtures. The solubility and staining reactions of the granules indicates that they may be an albumino-fatty compound or mixture, possibly an albumino-lipoid. There is no reason to suppose that the granules in muscle fibers are fundamentally different chemically from granules to be demonstrated by similar methods in other tissues of the body. It is reasonable to suppose that the true interstitial granules of muscle fibers are subject to some variation chemically in different species and under varying nutritive conditions.

b. Chemical nature of fat droplets

The fat droplets of muscle fibers are mentioned by many observers but few have attempted to determine the exact chemical nature of the fat. Usually, it seems, the droplets have been looked

upon as neutral fat. As already mentioned, Knoll ('80, '81, '90) thought the true interstitial granules to be composed, in part at least, of lecithin, but he too considered the fat droplets to be neutral fat. Bell ('10) holds that neutral fat droplets in muscle fibers are readily stained by simple alcoholic solutions of Scharlach R, but many 'liposomes', which are not so highly refractive as neutral fat droplets and consist wholly or in part of lipoids, can be stained only by alkaline Scharlach R (Herxheimer's method). Bell ('11) thinks the liposoms consist mainly of olein together with some low-melting fat other than olein. He states that many faintly-refractive liposomes which do not stain readily with simple alcoholic solutions of Scharlach R or with osmic acid, are stained somewhat faintly by Herxheimer's method and are composed in part of a substance other than fat, possibly an albumino-lipoid. Liposomes which stain faintly by Herxheimer's method and contain a non-fatty element are believed by Bell to be of most common occurrence in the muscle fibers of poorly nourished individuals.

1. *Refractive character: Double refraction.* The fatty droplets of muscle fibers may be seen in fresh tissue to which no foreign substance has had access. Preparations are made by rapidly teasing the fibers on a slightly warmed slide and applying a cover glass with slight pressure. The droplets present the highly refractive appearance characteristic of fat droplets and must be regarded as pre-existing bodies, that is to say they are not produced by histological reagents. Fat droplets are well brought out in fresh preparations mounted in normal saline. They vary somewhat in refractive index in different individuals but usually the variation in a single preparation is not pronounced. The true interstitial granules may also be observed in such preparations. These granules likewise vary somewhat in refractive index but are usually less refractive than the fat droplets. Judging merely from refractive index certain granules may be classed as either faintly refractive fat droplets or highly-refractive true interstitial granules. Preparations mounted in 2 to 5 per cent potassium hydroxide show the fat droplets very clearly for an hour or more but the true granules disappear almost immediately.

Bell ('11) states that the fat droplets of muscle fibers are all isotropic. I have examined only a few specimens with the micro-polariscope. The droplets were always singly and not doubly refractive. This shows that the droplets are not a fat which is fluid crystalline in form, such as the cholesterine compounds.

2. *Solubility.* The fat droplets of muscle fibers are readily soluble in cold absolute alcohol and in ether. Ninety-five per cent alcohol usually dissolves the droplets from frozen section or teased preparations in a few minutes. Tissue fixed in seventy per cent alcohol frequently shows a gradual diminution of the quantity of fat. It is well recognized that tests of solubility are of little value in determining the chemical character of fats in the tissues, especially as such fats, at least in most cases, are not in a pure state but exist as mixtures. Neutral fat has usually been considered insoluble in 70 per cent alcohol. However the fat droplets of muscle fibers, having a diameter of but 1 to 3 μ , must be regraded as in an extremely fine state of division, thus favoring prompt solution and, moreover, the quantity of solvent is very many times that of the fat dissolved. The fact that fat droplets in muscle fibers are sometimes dissolved by 70 per cent alcohol does not prove that they are not neutral fat.

3. *Results with Scharlach R and Sudan III.* Bell ('10) states that I had shown clearly the great superiority of alkaline alcoholic solutions of Scharlach R and mentions that my results had not yet been published. My observations concerning the staining of fat droplets in muscle fibers with alkaline alcoholic solutions of Scharlach R and Sudan III and with simple alcoholic solutions of the same dyes, were made in the Laboratory of Anatomy of the University of Missouri three years ago and are here given in a corrected form. At that time I observed the position of fat droplets in muscle fibers, a subject already discussed in this paper.

Scharlach R and Sudan III are usually employed as saturated solutions in 70 to 80 per cent alcohol. Such solutions frequently fail to stain the fat droplets of muscle fibers. The best results are obtained by heating the alcohol at the time of preparation of the stains or by permitting a certain amount of evaporation during the staining process. Even after an application of twenty-

four hours, these stains may color only a small part of the total number of fat droplets which can be seen in fresh preparations or demonstrated with Nile blue. Herxheimer ('04) does not state specifically that his alkaline-alcoholic solution of Scharlach R will color any fat droplets which cannot be stained with simple alcoholic solutions but he quotes Erdheim ('03) as having made such a claim. In so far as I have observed, alkaline-alcoholic solutions of Scharlach R and Sudan III stain all the fat droplets of muscle fibers. Herxheimer's stain usually gives a deep red color to droplets faintly stained or left colorless by simple alcoholic solutions. As already stated, true interstitial granules are not stained by Sudan III and Scharlach R. Frozen sections or teased preparations of muscle fibers, as well as of other tissues, which, when stained by the ordinary stock solutions of Sudan III and Scharlach R in 70 per cent alcohol may appear fat free, are sometimes shown to be crowded with fat droplets when examined in the unstained condition or when stained with Herxheimer's stain, or with Nile blue followed by immersion in an alkaline medium. In some specimens of muscle the simple alcoholic solutions stain all the fat droplets which can be seen in the fresh tissue.

The fact that fat droplets in muscle fibers are frequently left unstained by the less concentrated solutions of Sudan III and Scharlach R does not seem to offer sufficient proof that such droplets are not neutral fat. Fat in adipose tissue of mammals which presumably is neutral fat, is occasionally colored so faintly by these stains that were it in finely divided droplets it would be almost colorless. I do not share the belief advanced by Bell that we must suppose the droplets to contain an admixture of albumin or other non-fatty substance. The droplets in the muscle fibers of emaciated individuals, in so far as I have observed, stain with as great intensity by Herxheimer's method as do those of well nourished individuals. The intensity with which droplets are stained both with Herxheimer's stain, and with simple alcoholic solutions of Scharlach R depends as much upon the conditions under which the dye is used as upon the nature of the fat. The fact that droplets stain faintly cannot in itself be taken as sufficient proof that they contain a non-fatty element.

4. *Osmium tetroxide.* It is well known that osmic acid is reduced only by the unsaturated fats, the reduction depending upon the oxidation of the fat. Therefore it has been considered that fat droplets that are blackened by osmic acid consist, wholly or in part, of unsaturated fats. However, unsaturated fats present in small amounts as mixtures with saturated fats may fail to blacken with osmic. The fat droplets in muscle fibers of many animals do not reduce osmic acid. While in certain individuals the fat droplets blacken with osmic acid, in other individuals of the same species the reduction does not occur. This shows that the unsaturated fat in the droplets is variable in amount.

5. *Results with the Nile blue method.* Smith ('07) explained the staining of fats with the basic anilin dyes as depending upon the formation of color-soaps by the action of fatty acids and color bases. Neutral fat, as such, can not be stained by basic anilin dyes, but after hydrolysis the free fatty acid combines with the color base. This observer also found that neutral fat in the tissues is in a very unstable condition, being hydrolyzed by dilute acids and even by the carbon dioxide of the air. Thus neutral fat droplets in stained sections, after having been hydrolyzed into fatty acid and glycerine by exposure to the air were observed to take the color of the basic stain. Smith found that Nile blue sulphate initially gives a red color to both neutral fat and fatty acid. Neutral fat retains the red staining quality but subsequently fatty acids form color-soaps with the Nile blue base, the deep blue of the soap obscuring the comparatively faint red color of the fatty acid. Aschoff ('09) found that the phosphatid lipoids and cerebroside are also colored blue by Nile blue sulphate. McCrae and Klotz ('10), who used Nile blue on sections of fatty liver, state that they experienced some difficulty in interpreting their results. According to Klotz ('09) "The blue coloration obtained in staining sections with Nile blue is not to be depended upon as indicating in every instance the presence of fatty acids, as the shade of the color is influenced by external circumstances." He, however, does not specifically state by what circumstances the color is influenced.

Nile blue has been used as a means of investigating the chemical nature of fat droplets in tissue sections, but in so far as I know

the method has not been applied to muscle fibers. I have followed directions given by Smith and Mair ('11) for the use of Nile blue sulphate but the results have not been very satisfactory. Apparently the fat droplets are decolorized by the 2 per cent acetic acid used as a differentiating fluid. The method which I have used with best success is given here under the heading "Material and methods." In brief it consists in setting free the color base by the addition of a small amount of alkali to the medium in which the section is mounted, or by washing in slightly alkaline water after differentiating in distilled water. Precipitates are formed if sections are not carefully washed before being placed in the alkaline solution. With a little care in making the preparations with Nile blue chlorhydrate, and apparently with the sulphate also, it is possible to stain all the fat droplets to be found in muscle fibers. That is to say, Nile blue will stain droplets that are not stained by simple alcoholic solutions of Sudan III and Scharlach R or by osmic acid. With Nile blue, as with other stains, fresh tissue should be used if all the droplets are to be stained, for they frequently disappear in fixed tissue.

As has already been said, Nile blue sulphate stains not only the fat droplets but, in formalin fixed material at least, it also stains the true interstitial granules. This is best shown by staining a section from the pectoral muscle of a pigeon or bat. The true interstitial granules are especially abundant and stain a bright blue, the shade depending upon the length of time in the stain and upon the reaction of the mounting medium. The fat droplets which may also be present in large numbers, usually stain a somewhat faint red but sometimes in various shades of purple or blue. In formalin fixed specimens of material containing large true interstitial granules, it is not difficult to distinguish the latter from fat droplets since the granules do not have the globular form of the droplets and for the most part present color differences.

The color assumed by the fat droplets when stained with Nile blue is of some importance as it may help in identifying the fats chemically. The color of the droplets depends, to a considerable extent upon the time occupied in staining and upon the alkalinity of the solution to which the stained section is exposed, as well as

upon the length of time left in this second solution. The red staining takes place rapidly. The blue staining is sometimes well marked after fifteen minutes but may not appear for several hours or may not occur at all. The color is also influenced by the reaction of the mounting medium. In any alkaline medium the pale blue droplets tend to become red, while in an acid medium the red droplets tend to become blue. The entire mass of droplets may stain uniformly either red, blue, or in various shades of purple. On the other hand, a droplet colored red may include within its mass one or several clearly marked smaller droplets which stain an intense blue. Again, the periphery of a droplet may show blue staining while the center is red. After preparations have been mounted for a variable number of days, droplets which at first were red may assume an intense blue color. In other preparations the red color is retained for months. I have observed a certain amount of blue staining of the fat droplets in every species of animal from which material was taken, including man. The blue staining compound is found in muscle fibers taken from well nourished animals as well as in fibers from animals poorly nourished.

It is difficult to interpret the color reactions of Nile blue upon the fatty substances of muscle fibers. The blue color of fat droplets may possibly indicate that they are, in part, either fatty acid or a lipoid substance, while the red color indicates the presence of neutral fats. However, the blue color of the fat droplets may, as we have seen, indicate the presence of a neutral fat which is easily hydrolyzed, the fatty acid then forming a color soap. When the blue staining takes place only after sections have been mounted several days, it is, in all probability, dependent upon the gradual hydrolysis of neutral fat. With fresh tissues treated with the dye for a few minutes only, the blue staining, which is sometimes immediately apparent, may be due to similar changes in the neutral fat. It is possible that blue staining of fat droplets in fresh unfixed tissue or in tissue obtained some time after the death of the animal, may be due, in part, to the solution in the fat droplets of the blue staining substance of the true interstitial granules. It was pointed out by Smith ('07) that fat droplets in

tissues may be hydrolyzed by the action of the formalin fixatives. It follows that in formalin fixed material the blue staining with Nile blue is often an expression of neutral fat which has undergone hydrolysis.

The fat droplets of the muscle fibers of many individuals of a species show no blue staining after a prolonged exposure to the stain. From this we may conclude that the droplets in these individuals contain little or no free fatty acids, phosphatid lipoids, or cerebroside. In no case, in so far as I have observed, does the staining with Nile blue afford convincing proof that any substance other than neutral fat is normally present in the fat droplets of muscle fibers during the life of the animal. Contrary indications may be due to postmortem changes.

6. *Results with the methods of Benda, Fischler and Klotz for free fatty acids and soaps.* According to Benda, neutral copper acetate forms, with free fatty acids, colored copper soaps which for the most part are insoluble in fat solvents. The methods of Benda, Fischler and Klotz depend upon this reaction. Fischler ('04) found that the fatty acid copper compound forms a lake with hematoxylin. He also stained soaps in the tissues by the same methods, the soluble potassium and sodium soaps being first converted into insoluble calcium soaps by the action of calcium salicylicum. Klotz ('06) suggested further modifications. Bell ('11) used the above methods on preparations from a considerable number of muscles, but in no case was able to get the color reaction for free fatty acids or soaps. He concludes that the liposomes (fat droplets) of muscle fibers do not contain either fatty acids or soaps. I have used these methods only to a limited extent and my results are in agreement with those of Bell. However a positive result with the methods of Fischler and Klotz should not be taken as certain proof of the existence of free fatty acid during life. The fixative employed in both these methods contains formalin and in the method of Klotz, acetic acid is added. It is thus possible that the staining occasionally depends upon the previous hydrolysis of neutral fat.

7. *Results with the Weigert method and related methods.* We have already discussed these methods and seen that they give

positive results with the true interstitial granules. The fat droplets of muscle fibers are not easily rendered insoluble by the action of chromic compounds. For the most part the droplets are dissolved by the alcohol or xylol used in the paraffin process even when the tissues have been treated with 10 per cent potassium bichromate for several days at 37°C. It follows then that the fat droplets in muscle fibers are not shown by the Weigert or iron-hematoxylin methods as ordinarily employed. When chrom-osmic fixatives are used, as in the methods of Altmann and Benda, the droplets are sometimes blackened and rendered insoluble, but usually they are still soluble. When, as rarely happens, fat droplets are rendered insoluble by the bichromate-osmic mixtures, and at the same time not blackened by osmic, they may be stained by Benda's iron-alum Krystallviolett and probably also by hematoxylin or iron-hematoxylin. Since the investigations of Smith and Mair ('08, '10, '11), Aschoff ('09), and of Fauré-Fremiet, Mayer and Schaeffer ('10) have shown that the phosphatid lipoids, combinations of cholesterin and fatty acids, as well as cerebroside, are rendered insoluble by the action of potassium bichromate, we may conclude that the fat droplets of muscle fibers do not, to any very considerable extent, consist of these fats. Saturated neutral fats are not rendered insoluble by the action of potassium bichromate and triolein is acted upon only very slowly. The fat droplets of muscle fibers not being readily acted upon by potassium bichromate, react as if they were composed wholly or largely of neutral fat.

8. *Formalin fixation.* Bell ('10) pointed out the fact that fat droplets in muscle fibers and other tissues are frequently not preserved by formalin fixation. Droplets of ordinary neutral fat he states are not affected in their staining by formalin fixation, but many faintly-refractive fat droplets, consisting wholly or in part of lipoids, cannot be stained after formalin fixation. The faintly-refractive droplets are either removed or rendered invisible by fixation. He finds that the action of the formalin fixative in one tissue may be unappreciable for weeks and in another nearly all the liposomes may be removed in a few minutes. He states that the varying effect of the fixative is probably due to the vary-

ing chemical composition of the liposomes. Bell also found that usually the fat droplets in the muscle fibers of adult and well nourished animals are less affected by formalin than are the droplets of young and poorly nourished animals. However, many exceptions to this rule were mentioned. Bell ('11) confirms his former observations and states further that the faintly-refractive liposomes, which are removed by formalin fixation, consist in part of a substance other than fat, possibly an albumino-lipoid. He believes that the disappearance of the liposomes is probably due to autolysis.

I have repeatedly observed the gradual disappearance of fat droplets from tissue fixed in formalin. However, I am not certain that neutral fat is not affected by formalin fixation. Smith ('11) in fact has found that ordinary formalin solutions hydrolyze the fat droplets of frozen sections which are kept in the fixative. I have frequently but not invariably found that the blue staining of fat droplets with Nile blue is more marked in formalin fixed material. Free fatty acids are soluble in Herxheimer's staining solution and hence cannot be demonstrated by Herxheimer's method. The fat droplets of formalin fixed material frequently disappear, not being visible either as crystals or as droplets staining blue with Nile blue. This indicates that the final change which takes place in the fat is not to be explained on the basis of simple hydrolysis. I believe, however, that the initial change may be hydrolysis of an unstable neutral fat. The fat droplets of the muscle fibers of the ox fetus, for the most part, stain red with Nile blue but after several days the droplets in the stained section assume a blue color. This change of color indicates that the fat is in an unstable condition and can be readily hydrolyzed. The fat droplets of the fetal tissue were not permanently preserved by formalin fixation. The observations of Bell and Smith, as well as my own, are based on the use of ordinary commercial formalin. This solution contains variable quantities of formic acid and possibly acetic acid. The hydrolysis of the fat may depend upon the presence of these impurities. It is thus possible that the variable action of formalin is to be explained in part by the variable chemical composition of the fixative.

The action of formalin on the fat droplets is not what we should expect if the droplets were an albumino-lipoid as suggested by Bell. In fresh unfixed muscle fibers the droplets readily coalesce to form larger globules. In formalin fixed material the coalescence is not so apparent, for the substance immediately surrounding each droplet has been coagulated. However by the examination of carefully teased muscle fibers it is easy to convince oneself that the droplets themselves are not hardened but may still coalesce. If albumin were present to any considerable extent, the droplets would be fixed and no coalescence would take place. Fauré-Fremiet and his collaborators have shown that albumino-lipoids are coagulated by formalin in such a manner as to render the fatty substance almost insoluble in fat solvents. The fat droplets of formalin fixed muscle fibers are apparently as easily dissolved by alcohol as in fresh tissue.

9. *Summary.* The fat droplets of muscle fibers are not largely composed of fatty acids or soaps for they do not stain by the methods of Benda, Fischler and Klotz. They are not fatty acids for they do not stain readily with basic anilin dyes. The fat droplets do not contain cholesterin esters to a very considerable extent for they do not present the characteristic anisotropic fluid-crystalline form. The droplets are not phosphatid lipoids or cerebroside for these substances are easily rendered insoluble by potassium bichromate, whereas the fat droplets of muscle fibers are not rendered insoluble. Also phosphatid lipoids and cerebroside stain with basic dyes, giving blue with Nile blue, while the fat droplets of muscle, at least for the most part, stain red with this dye and are colored blue only after a chemical change has occurred in the fat. Fat droplets in muscle fibers are readily stained by alkaline-alcoholic solutions of Scharlach R and Sudan III but are frequently left unstained by simple alcoholic solutions of these dyes. The evidence which tends to show that the fat droplets of muscle fibers are neutral fat (glycerin esters of fatty acids) is largely of a negative character. It is improbable that they are pure neutral fat, yet it may be said that no certain proof has yet been offered that any substance other than neutral fat is present in the fat droplets of muscle fibers during the life of the animal.

VI. PHYSIOLOGICAL SIGNIFICANCE OF INTERSTITIAL GRANULES AND FAT DROPLETS

Physiological significance of true interstitial granules

Several investigators have held that true interstitial granules give origin to fat droplets either by a fatty metamorphosis or by serving as a focus about which fat is deposited. Kölliker ('88-89) believed that fat droplets in muscle fibers arise from true interstitial granules by a process of fatty metamorphosis and Schaeffer ('93) advanced similar views. Holmgren ('10) states that the deposition of fat in muscle fibers is apparently influenced by the true interstitial granules but he thinks the granules are actually transformed into fat only under exceptional or pathological conditions. Altmann ('94) held that his bioblasts (true interstitial granules) are not transformed in toto into fat but act as a focus within and around which fat is accumulated. Arnold has advocated similar views. Bell ('11) does not believe that granules which stain with acid fuchsin (Altmann's granules) give origin to fat and he is of the opinion that true interstitial granules do not occur in vertebrate muscle. His conception of the deposition of fat in muscle fibers is nevertheless essentially in accord with the theory advanced by Altmann. Bell holds, namely, that the fat of muscle fibers is deposited as 'liposomes' around a pre-existing non-fatty body, possibly an albumino-lipoid. As we have seen, Altmann's granules are probably an albumino-lipoid formation and conversely albumino-lipoids may be expected to stain with Altmann's acid fuchsin.

The reader is referred to the literature for a presentation of the arguments offered by various authors in support of the idea that true interstitial granules give origin to fat droplets or serve as foci about which fat is deposited. It may be said that, at present, the truth of such a conception is not sufficiently demonstrated to warrant us in believing that there is a genetic relationship between true interstitial granules and fat droplets.

According to Holmgren ('07, '10) the colorable substance (method of Benda) of the interstitial granules is necessary to the proper functioning of the contractile elements. During the

latent period, substance from the granules was thought to pass into the muscle columns there to be used in the stage of active contraction. The examination of insect and vertebrate muscle fibers, by the various methods employed in this study, has afforded little in support of Holmgren's views concerning the physiological significance of the granules.

Knoll ('80) and Knoll and Hauer ('92) found that the true interstitial granules are not removed in inanition. Feeding experiments with a dozen white rats lead me to conclude that the alcohol-soluble portion of the true interstitial granules which stains with Cresylviolett is increased in amount when rats are heavily fed and decreased when the animals are kept on low rations. This may indicate that the alcohol-soluble substance is of metabolic importance and such an assumption would seem the reasonable one.

Before dismissing the subject of the physiological significance of true interstitial granules, it should be mentioned that Arnold ('09) believes that glycogen is bound to the sarcosomes (true interstitial granules), and Kingsbury ('12) thinks that mitochondria, in general, act as a reducing agent and in certain cases may be concerned in exercising the function of cell respiration.

Physiological significance of fat droplets

The presence of fat droplets in striated muscle fibers has been regarded by Van Gehuchten ('89) and others, as of pathological significance. Probably this is still the prevailing opinion with respect to cardiac muscle. Schaeffer ('93) believed that fat droplets in the skeletal muscle fibers of vertebrates may occur under normal conditions but are usually pathological. Walbaum ('99) found that fat droplets were of very frequent occurrence in normal human muscle fibers but thought the quantity of fat bore no direct relation to the nutritive condition of the individual. Kölliker ('88) regarded the fat droplets of insect muscle as reserve food material. Knoll and Hauer ('92) found that fat droplets in the muscle fibers of pigeons are removed by starvation but the true interstitial granules are not removed. Krause ('11) states that the fat droplets in muscle fibers are not independent of the

nutritive condition of the animal. Bell ('11) found that the liposomes (fat droplets) of the striated muscle fibers of rats were entirely removed when the animal was starved until it had lost 25 per cent or more in body weight. During starvation, the liposomes gradually became faintly refractive and decreased in size, number and in staining intensity with Herxheimer's Scharlach R and with osmic acid. When the starved animal was again given food the liposomes gradually reappeared, increasing in size, number, refractive power, and staining intensity as the animal gained weight. In normal rats which were fed on fat meat for several days, the liposomes were greatly increased in number, size, and staining intensity. When summer frogs were fed heavily on olive oil or fat meat, there was a striking increase in size, number and staining intensity of the liposomes. No changes were produced in the liposomes by the feeding of grape sugar, starch, palmitic acid, sodium oleate, or lean meat. Since the liposomes stained faintly when they first appeared, Bell supposed that they then contained a relatively small percentage of fat together with some substance other than fat, possibly an albumino-lipoid. He regarded the liposomes as foci where fat is deposited and concluded that they consist of reserve food substances mainly, at least, in the form of fats.

In this connection I have examined the muscle fibers of a dozen white rats on various nutritive planes. Figure 1 represents fibers from the pectoralis major of an adult rat which had been heavily fed on fat meat for seven days. The fat droplets were stained with Herxheimer's Scharlach R. Figure 2 shows muscle fibers from the pectoralis major of a rat which had been kept for ten days on short rations of a fat free diet consisting mainly of cellulose. Fat cells were almost completely absent from the subcutaneous tissue and mesentery of this animal. Fat droplets are practically absent from the light fibers as shown in the figure, while dark fibers have a smaller quantity of fat than is normally present even in light fibers. In the diaphragm of this animal, the quantity of fat, although greatly reduced from the normal, was somewhat greater than that found in the pectoralis major.

The amount of fat in the cardiac muscle fibers of emaciated rats was less than in fibers of well nourished individuals. Animals

fed on fat meat showed an increased amount of fat in the cardiac fibers but the increase was not so great as in skeletal muscle. The muscle fibers of the pectoralis major of normal rats kept on a diet of bread and lean meat show fat intermediate in amount to that represented in figures 1 and 2. In several rats the superficial fibers of the pectoralis major contained less fat than those somewhat removed from the surface. The fibers illustrated in the figures were not superficially placed. After rats have been fed on fat meat for a few days, the quantity of fat in the muscle fibers appears to have been increased to the maximum. Further feeding increases the amount of connective tissue fat but seems to have no effect upon the fat in the muscle fibers. As already mentioned, dark muscle fibers are more clearly marked in well nourished animals than in emaciated animals of the same species.

The fat droplets of muscle fibers are clearly to be regarded as reserve foodstuff. The work of Bell in this respect is so convincing as scarcely to require confirmation. I have not made observations on the effect of starch, sugar or protein diets, as has Bell.

VII. SUMMARY AND CONCLUSIONS

The interstitial granules of striated muscle may be classed as true interstitial granules and fat droplets as was done by Kölliker. Both the granules and fat droplets are factors in bringing about the dark or cloudy appearance of muscle fibers.

The two types of fibers, dark and light, the occurrence of which is well known in adult vertebrates, are also present in the muscles of the fetus.

The terms "red and white muscle" refer to macroscopical color differences only. Applied to the microscopic appearance of muscle fibers these terms become a misnomer if used as synonymous with "dark and light muscle fibers."

The interstitial granules and fat droplets of muscle are somewhat uniformly arranged in longitudinal and in transverse rows between the muscle columns. Small granules and fat droplets form transverse rows in segment *J* on either side of the membrane of Krause while those of larger size are in segment *Q*. The

arrangement of the granules and fat droplets within muscle fibers is not dependent upon any connection with a fibrous net-work but is determined solely by the position of the membrane of Krause, the size of the granules, and the pressure of the muscle columns.

The true interstitial granules are of a soft, plastic substance and have no limiting membrane.

The exact chemical nature of the true interstitial granule is unknown. They are certainly not composed wholly of fat, though they contain an alcohol-soluble element. As was suggested by Regaud, they may be an albumino-lipoid.

Many fat droplets in muscle fibers are not preserved by formalin fixation. After a variable length of time in formalin fixatives the droplets may disappear. Fresh tissues must be used if all the droplets are to be demonstrated. This confirms a conclusion drawn by Bell.

Fat droplets in muscle fibers are frequently stained but faintly or left colorless by the commonly used solutions of Scharlach R and Sudan III in 70 per cent alcohol. Alkaline-alcoholic solutions of Scharlach R applied to fresh tissue stain all the fat droplets of muscle fibers. By this method preparations may sometimes be shown to be loaded with fat droplets when none are stained by the simple alcoholic solutions.

Nile blue sulphate and Nile blue chlorhydrate color all the fat droplets of muscle fibers when fresh tissue is used and stained sections are placed in alkaline water or mounted in an alkaline medium. The droplets are usually colored red, but under certain conditions they stain blue. With favorable material, pectoral muscles of pigeon and bat, after a short formalin fixation, both true interstitial granules and fat droplets may be stained in the same preparation, the former blue, the latter red.

Many fat droplets of muscle fibers are not blackened by osmium tetroxide.

For the most part the fat droplets of muscle fibers are neutral fat, glycerin esters of fatty acids. No convincing evidence has yet been presented to show that the fat droplets contain any substance other than neutral fat. They may not be pure neutral fat but it is improbable that they contain any considerable amount of albumin or other non-fatty substance.

Both true interstitial granules and fat droplets are of wide distribution in striated muscle, occurring under normal physiological conditions both in insect muscle, and also in the skeletal and cardiac muscle of vertebrates.

The physiological significance of the true interstitial granules is uncertain.

The quantity of fat in muscle fibers is increased when the animal (rat) is fed on fat meat and decreased during inanition. The fat droplets of muscle fibers are reserved food material. This conclusion was reached by Bell.

This study was conducted under the direction of Prof. Irving Hardesty and I am under obligation to him for many helpful suggestions. I am also indebted to Prof. Gustav Mann who has given me valuable assistance.

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ON THE FATE OF THE JUGULAR LYMPH SACS AND THE DEVELOPMENT OF THE LYMPH CHANNELS IN THE NECK OF THE PIG

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FOUR FIGURES

In a study of the morphological changes which the jugular lymph sacs and the lymph channels in the neck of the embryo pig undergo during development, a number of questions must be considered. What are the primary lymph channels? Are they characteristic and constant in form? How are they modified during development? What is the correlation between the earliest lines of drainage and the drainage found in the adult? What are some of the factors controlling these transformations? These and other questions arise. The purpose of the following paper is to make an analysis, and to offer a few suggestions on the points mentioned above.

The undertaking of this work was suggested by Dr. Sabin, and it was through her kindness that this study was possible. There have been accumulating in the laboratory from previous studies a number of injections of lymphatics in embryo pigs of all stages made by Dr. Sabin. These with numerous new injections have been cleared by the Spalteholz method,¹ and the present paper is based on a comparative analysis of these specimens. It has been the aim to give as accurately as possible the location of the lymphatics and the morphological changes in successive stages of development. There has been no attempt to describe the minute structure of the lymphatics but simply to trace the gross changes in the lymph channels.

¹ Spalteholz, W., Ueber das Durchsichtigmachen von menschlichen und tierischen Präparaten. Leipzig. Verlag von S. Hirzel. 1911.

The work of Sabin has shown that lymphatics first appear in the embryo pig 10 to 11 mm. long as an outbudding from the anterior cardinal veins opposite the third, fourth and fifth segmental branches. From these primitive buds a plexus of lymphatics is formed along the dorsolateral border of the anterior cardinal vein and this plexus is transformed into a non-muscular endothelial lined sac. From this primitive sac by continued centrifugal growth a large number of sprouts grow dorsalward into the posterior triangle of the neck and form a complete arch of lymphatic capillaries connecting at either end with the primitive sac. This entire arch of capillaries becomes transformed into a part of the jugular lymph sac which explains the form of the final sac as shown in figure 1. From the jugular sac, still by centrifugal growth, the peripheral lymph vessels radiate forward over the head and backward over the anterior part of the body forming plexuses which are characteristic and definitely located.²

For convenience I shall use the following terms in referring to the lymph sac, the form of which is shown in figure 1. (1) The anterior curvature of the lymph sac is the portion lying behind the pharynx against the internal jugular vein. (2) The sac stalk is the portion of the sac also on the internal jugular vein extending between the point where the valve develops at the junction of the internal and external jugular veins and the anterior curvature. This is the first part of the sac to develop. (3) The apex is the portion of the sac lying in the posterior triangle of the neck. The reasons for this division of the sac are not obvious in figure 1, but I shall show that they correspond to the function of the three different parts of the sac. The apex of the sac connects with the sac stalk both through the anterior curvature and more directly by a large channel which joins the stalk not far from the valve into the vein.

The form of the jugular sac is well shown in figure 1, which is a diagram made from an embryo pig 2.8 cm. long and in figure 2 which is a drawing of an injection of the lymphatics in a pig 3.5 cm. long. From the sac four groups of lymphatic vessels develop.

² A part of this work is in the *Amer. Jour. Anat.*, vol. 1, 1901-1902, and a part of it will be published in the *Ergebnisse für Anat. und Entwicklungsgeschichte*.

The first group consists of a few large vessels which have grown from the apex, the most superficial part of the sac, over the scapular region. The second place of growth is the dorsal border of

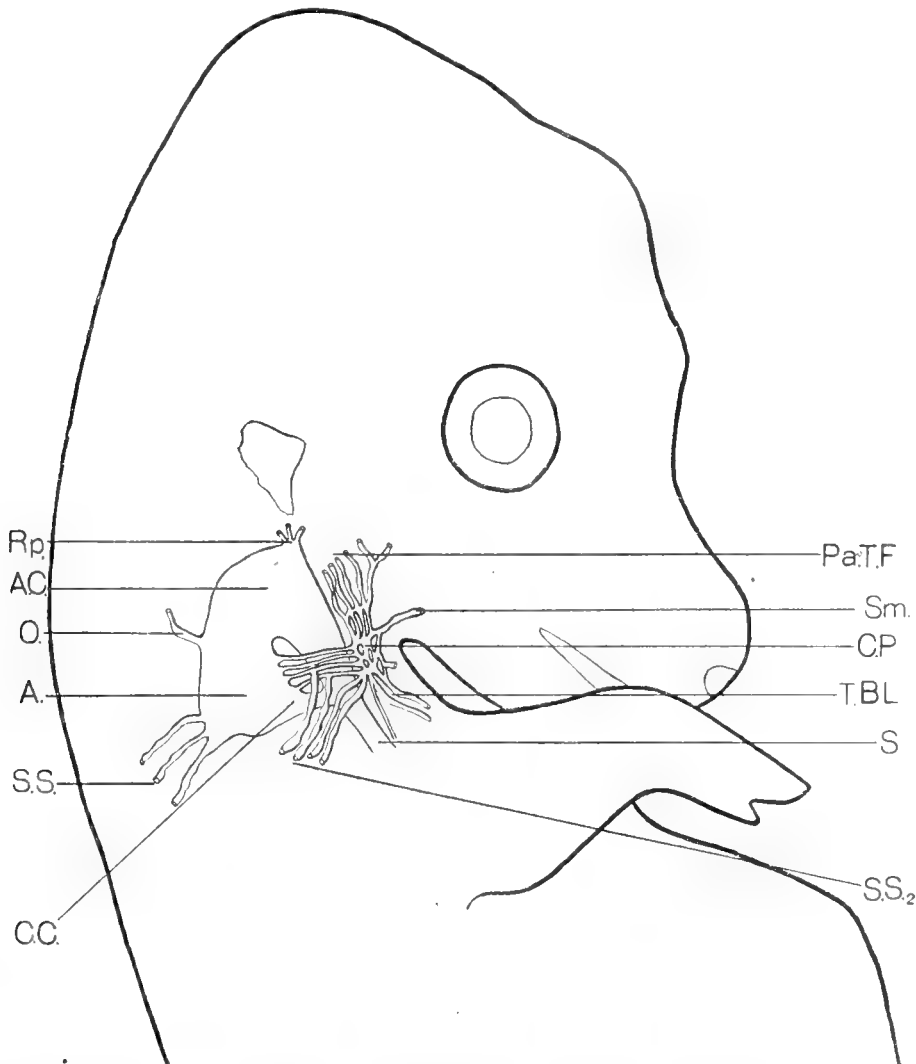


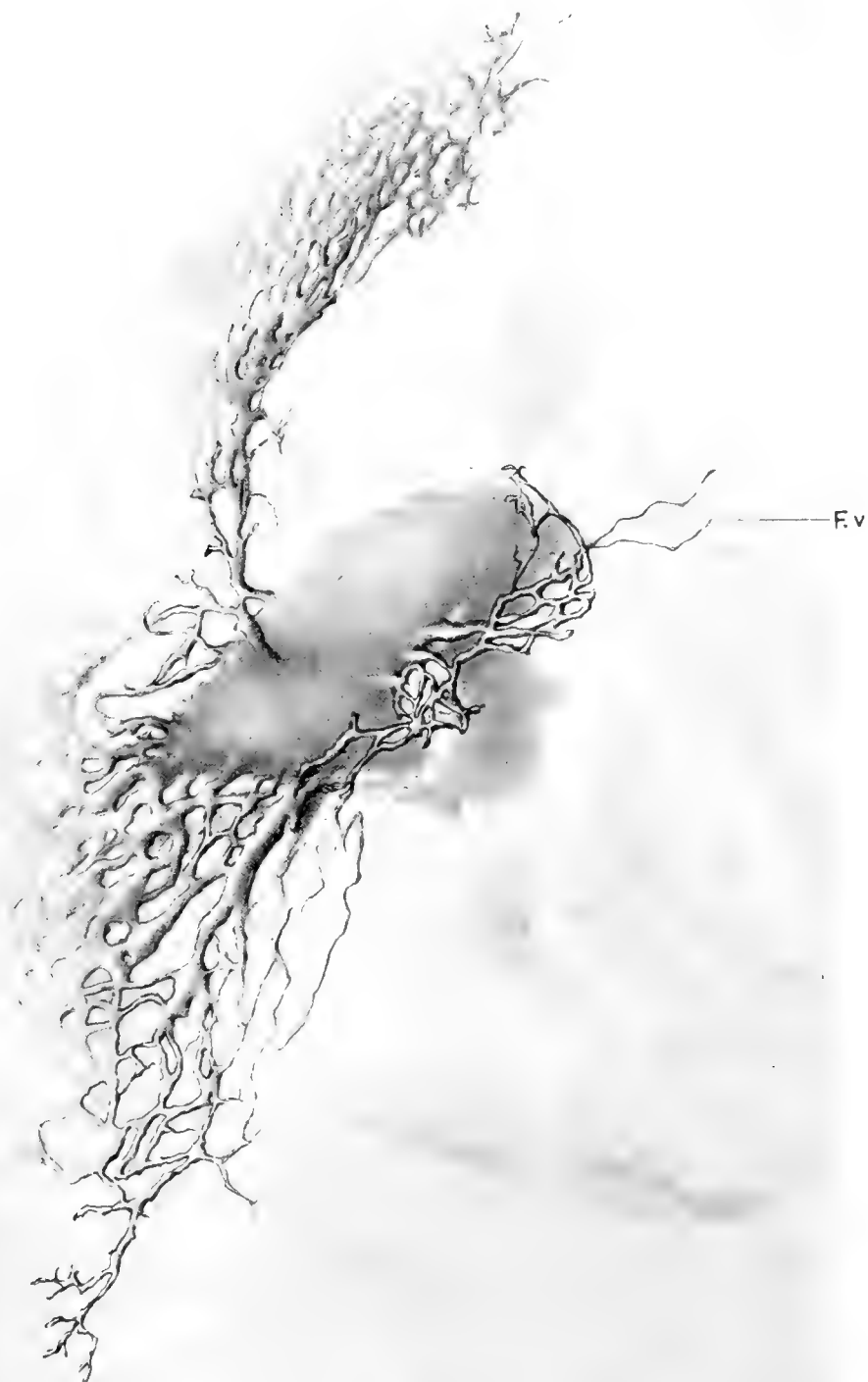
Fig. 1 Diagram of the jugular lymph sac in an embryo pig 2.8 cm. long to show the points of origin of the peripheral vessels. $\times 10$. *A*, apex; *A.C*, anterior curvature; *C.C*, cross connection between the apex of the sac and the sac stalk; *C.P*, superficial cervical plexus; *O*, occipital lymph duct; *Pa.T.F*, point of origin of the posterior-auricular, temporal and facial lymphatics; *Rp*, retropharyngeal lymphatics; *S*, stalk of the sac; *Sm*, submaxillary lymphatics; *S.S*, primary supra-scapular lymphatics; *S.S₂*, suprascapular lymphatics from the cervical plexus; *T.B.L*, thoracic and branchial lymphatics.

the apex just anterior to the suprascapular vessels. A large duct extends forward over the occipital region of the head. This particular vessel is very large in the human embryo as can be seen

in figures 505 and 506 of the *Handbuch der Entwicklungsgeschichte des Menschen*. Keibel and Mall., vol. 2, 1911, ps. 708–709, after Sabin. The third group of vessels is from the anterior curvature of the sac where it arches dorsalward and lateralward behind the pharynx. The importance of these vessels is shown both by their size and their early appearance. In an embryo 2.8 cm. long the anterior curvature has a distinct bulge protruding toward the buccal cavity, and in one specimen a few ducts can be seen radiating toward the pharynx. From this retropharyngeal process of the sac are to be developed all of the lymphatics of the pharynx, Eustachian tube, the nasal cavity and a part of those of the tongue. The fourth group of vessels is by far the largest. In figure 2 will be seen a group of vessels from the ventral border of the apex of the sac which grow ventralward external to the sterno-cleido-mastoid muscle and form an extensive lymphatic plexus along the course of the external jugular vein. This plexus I shall call the superficial cervical plexus since it gives rise to the superficial cervical lymph glands.

The injection shown in figure 2 is not a complete injection for these vessels. The point of injection was in the suprascapular vessels which is an indirect point for the superficial cervical plexus. The vessels from the ventral border of the apex of the sac are present in an embryo 18 mm. long and hence they begin at about the same time as the suprascapular lymphatics. The superficial cervical plexus as shown in figure 2 has already sent a group of vessels cranialward, part of which are shown as posterior auricular lymphatics. The vessels which grow forward along the external jugular vein divide into two groups, the temporal and the facial. From the ventral border of the superficial cervical plexus develop

Fig. 2 Injection of the jugular lymph sac in an embryo pig measuring 3.5 cm. long. Magnified about 10 times. This is the same specimen which was shown as figure 3 in *The American Journal of Anatomy*, p. 186, vol. 3, 1904. The specimen has since been cleared by the Spalteholz method so that it shows the relation of the superficial lymphatics to the jugular lymph sac. It is a complete injection of the suprascapular and occipital plexuses and an incomplete injection of the beginning cervical plexus. The sac stalk shows faintly where it extends internal to the arm. *F.v.*, facial vein; a little blood in this vein enables one to locate the position of the superficial cervical lymphatic plexus.



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as shown in figure 1 the lymphatics for the skin of the neck and the submaxillary vessels, while the caudal end of the plexus gives rise to the superficial lymphatics of the arm and of the thoracic wall. Thus the jugular lymph sac gives rise to the suprascapular, occipital and pharyngeal lymphatics directly and is the place of origin of the superficial cervical plexus which in turn supplies all the rest of the lymphatics for the head, face, neck, thorax and arm. The deep lymphatics of the arm have not yet been worked out in the pig. In the cat and in human embryos they arise from an extension of the jugular lymph sac which lies along the primitive ulnar vein.

These fundamental groups of lymphatics the suprascapular, occipital and superficial cervical, which can be seen in the embryo 3.5 cm. long and indeed can be injected a short distance from the lymph sac much earlier namely in specimens measuring 18 mm. are constant. Ducts originate from definite places and establish definitely-localized plexuses. Thus in the early stages there are distinct plexuses in the skin which are connected with each other only through their central connection with the sac. Such a primary plexus for example is the occipital plexus of figure 2. By subsequent development however, these separate areas become interconnected, so that an injection instead of being limited to one of the primary plexuses spreads out quite widely, reaching the sac not by a single set of ducts but by a number according to the extent of the injection. Thus, plainly, the earliest lymphatics drain definite areas which are distinctly located and definitely defined.

In figure 2 it is shown that the suprascapular vessels drain by a few vessels sometimes not more than one or two directly into the apex of the sac. At this stage there are a few small anastomoses between the suprascapular vessels and the superficial cervical plexus. These anastomoses are destined to become very abundant so that there are eventually more vessels which connect the suprascapular plexus with the superficial cervical plexus than with the primary sac.

This process of the development of anastomoses between the different primary plexuses goes on until the entire superficial

lymphatic plexus is a complete layer of lymphatics covering the body. This stage is shown in Sabin's figure 5 in *The American Journal of Anatomy*, p. 188, volume 3, 1904. In this figure it is not possible to analyze the primary plexuses; the suprascapular, occipital, posterior auricular, temporal, facial, cervical, thoracic and brachial vessels make one continuous plexus. When an injected specimen of this stage is cleared however by the Spalteholz method the place of origin for each plexus can be made out. In figure 3 it is clear that the primary lymph sac has the three divisions already given, namely, the apex in the posterior triangle the anterior curvature and the sac stalk lying deeper and hence showing very faintly on the internal jugular vein. The apex of the sac and the anterior curvature may now be called lymph glands, the sac stalk however remains as the deep jugular lymph trunks. In comparing figures 2 and 3 it is clear that in the earlier stage the outline of the sac is a comparatively smooth curve from the stalk around to the apex. In an embryo pig 20 mm. long the entire dorsal border of the sac has a series of sprouts but the permanent ducts are however limited to certain areas along the sac and these parts enlarge while the intermediate parts remain small. This determines the position of the lymph nodes.

Three factors seem to guide this primary node formation in the sac. First the apex of the sac receiving as it does the suprascapular and occipital vessels directly and all of the vessels of the face, neck, arm and thorax indirectly through the superficial cervical plexus is the largest center of drainage in the neck. Lymph glands develop at the centers of drainage and the apex of the sac therefore becomes an early and a large node. Second the portion of the sac between the apex and the anterior curvature can be assumed to be comparatively non-functional as a path for lymph conduction, for the apical drainage would most easily pass to the veins by way of the cross connection to the stalk. Hence the portion of the sac intervening between the apex and the anterior curvature remains small. Third, the development of the sternocleido-mastoid muscle which crosses the sac between the apex and the anterior curvature probably causes a pressure to be exerted at this point. Drainage, function and structural relations can

be said to be factors in controlling node formation and development both in the lymph sac and along the peripheral lymphatics. The relations of the peripheral lymphatics to the sac and the development of nodes thus described are constant.

The superficial cervical plexus needs a very careful description. It is clear that it is an important structure since it drains so large

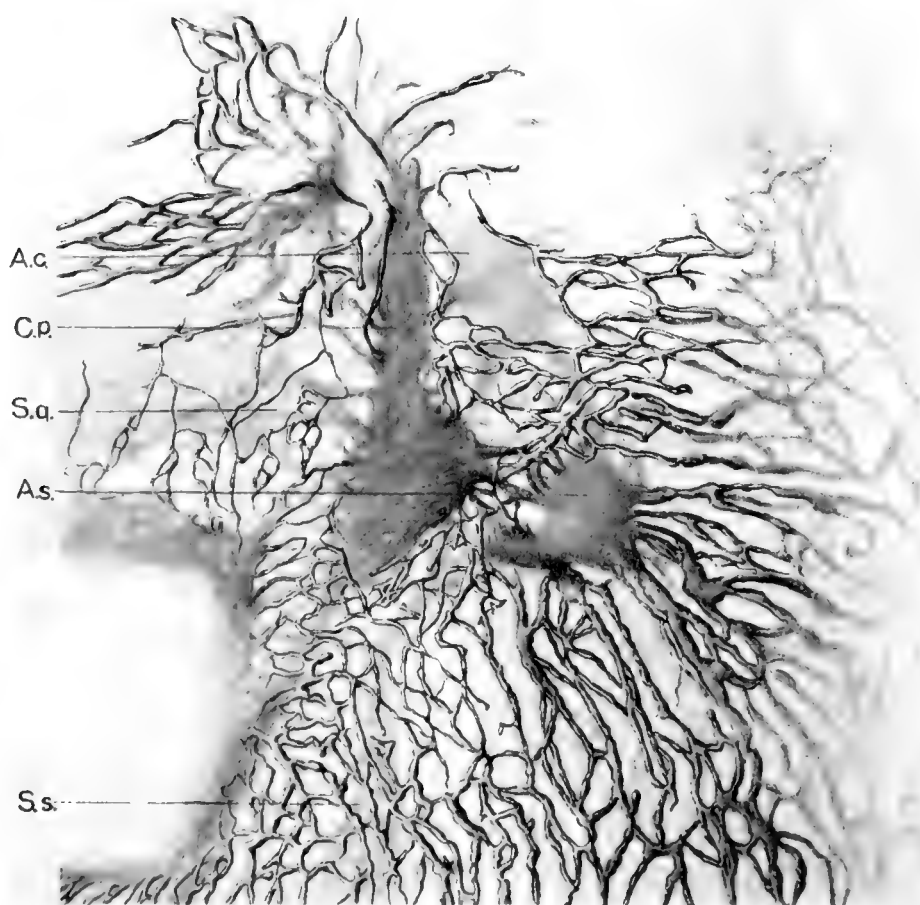


Fig. 3 Injected jugular lymph sac, superficial cervical lymph plexus and the peripheral lymphatics in the neck of an embryo pig measuring 5.5 cm. long. Magnified about 7.5 times. This figure is to be compared with figure 5 in *The American Journal of Anatomy*, p. 188, vol. 3, 1904, which is a complete injection of the superficial lymphatics of the same stage. *A.s.*, the apex of the jugular sac making the lymph gland of the posterior triangle; *A.c.*, anterior curvature of the lymph sac making the deep jugular lymph gland; *C.p.*, superficial cervical lymph plexus; *S.g.*, submaxillary lymph gland; *S.s.*, stalk of the jugular lymph sac.

an area. As seen in figures 3 and 4 it is an extensive and dense plexus of lymphatics lying along the external jugular vein lateral to the sterno-cleido-mastoid muscle. It has in reality two points of origin. First, the ventral border of the apex of the sac shown in figure 2 for an earlier stage but still better in figure 3. The second place of origin is a plexus of lymphatics from the stalk of

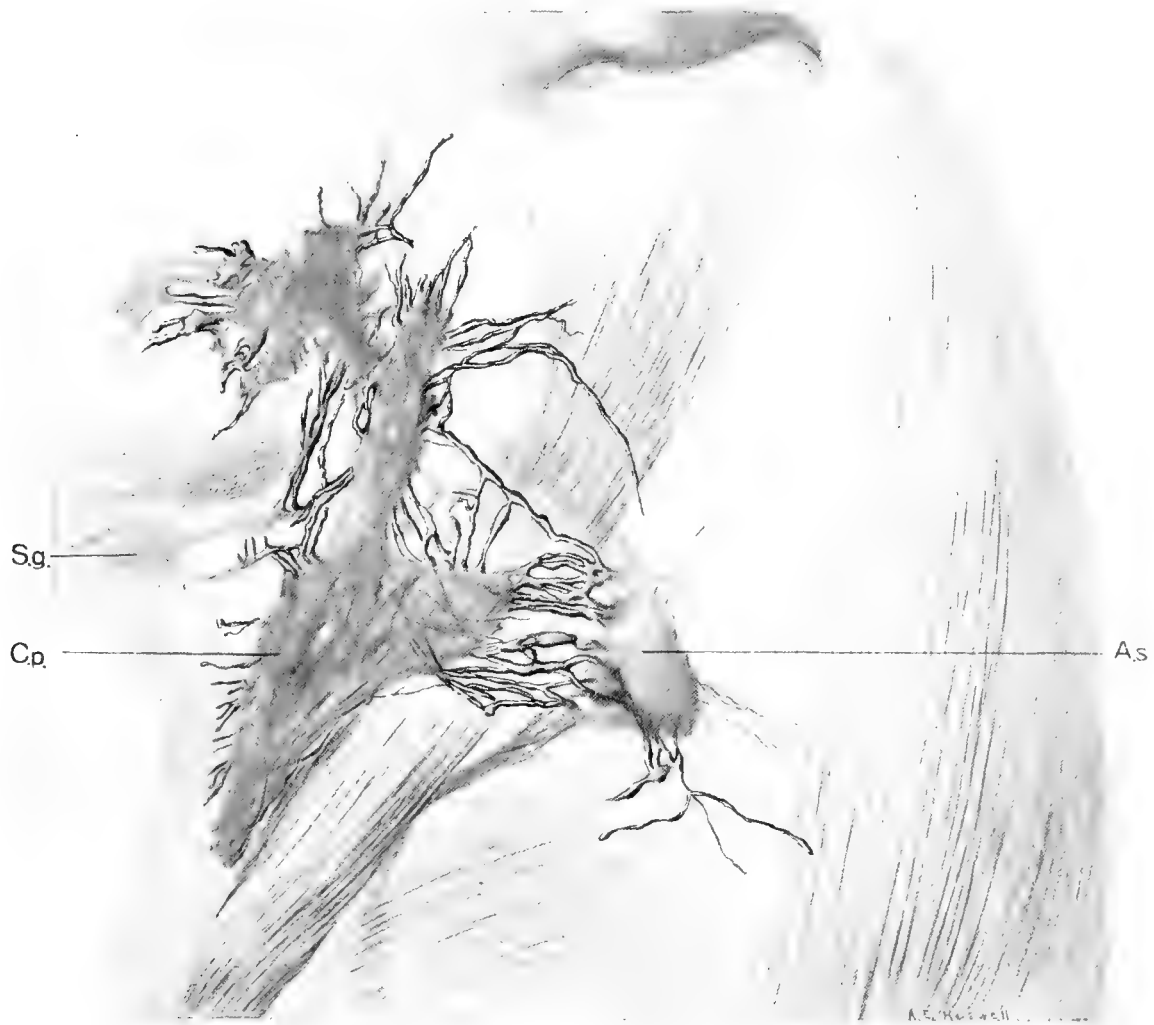


Fig. 4 Injected jugular lymph sac and cervical lymph plexus in a pig measuring 7.5 cm. long, to show the relation of the developing glands in the neck to the sac. Magnified 6.5 times. *A.s*, apex of the sac or gland of the posterior triangle of the neck. The anterior curvature of the sac, which is a deep jugular pharyngeal lymph gland, shows behind the sterno-cleido-mastoid muscle. *C.p*, superficial cervical plexus which is destined to be a group of lymph glands. At the cerebral end of the plexus is a developing facial gland. *S.g*, submaxillary lymph gland.

the sac which follow the external jugular vein. This group of vessels is indicated by one trunk in figure 1, but the vessels show much better in a mesial view. Mesial sections of pigs 5 to 6 cm. long show that there is an abundant plexus of vessels arising from the stalk of the sac in the root of the neck near the place where the lymphatic sac connects with the vein. A few of these vessels follow the stalk or deep jugular trunk and are mentioned in connection with the lymphatics of the pharynx, more of them however follow the external jugular vein and connect with or help to form the superficial cervical plexus. In one specimen, one of these vessels connects directly with the external jugular vein instead of with the sac stalk. Thus the superficial cervical plexus can be said to arise not only from the apex of the sac but from the sac stalk as well or even by a variation directly from the veins. The plexus of vessels which follows the external jugular vein is very conspicuous in injections of cat embryos where the vein is entirely surrounded by a plexus of lymphatics.

In the pig 5.5 cm. long the cranial end of the cervical plexus gives rise to three sets of lymphatics. First there are vessels growing behind the ear making a posterior auricular set. There is also a secondary plexus of vessels close to the main plexus which supplies (2) the temporal lymphatics and (3) the facial vessels. From the ventral border of the cervical plexus grow a group of deep submaxillary vessels and a very abundant plexus of vessels for the skin of the neck. The submaxillary plexus supplies the lower jaw and tongue and it has anastomoses both with the facial group and with the vessels from the anterior curvature of the sac. The submaxillary plexus may also connect with vessels along the internal jugular vein. The importance of these anastomoses between the primary groups of vessels cannot be emphasized too much.

The caudal end of the cervical plexus extends directly into the superficial thoracic vessels and supplies also the superficial lymphatics of the arm. These would show better in a ventral view than they do in the lateral one. In this point the drainage in the pig is different from that of the human embryo where the large thoracic vessels and the superficial vessels of the shoulder drain

into the axillary vessels as can be seen in the figures from the *Handbuch der Entwicklungsgeschichte* quoted above. The pattern of the superficial vessels of the arm is to be seen in figure 5 of *The American Journal of Anatomy*, p. 188, volume 3, 1904.

The mesial view is better for the lymphatics of the anterior curvature of the sac. They show faintly in figure 4. Three groups of vessels can be injected from the anterior curvature in specimens 5 to 6 cm. long. First an abundant group which extend to the wall of the pharynx, second vessels which extend along the sphenoid bone to the nasopharynx and third a small chain which grows outward toward the ear. It is probable that these are the lymphatics for the Eustachian tube. The anterior curvature is not the sole place of origin for the pharyngeal lymphatics for some injections show vessels which arise from the stalk of the sac low down in the neck, that is to say near the valve into the vein. These vessels follow the course of the sac stalk, along the internal jugular vein to the wall of the pharynx. Some of the pharyngeal vessels anastomose with the superficial cervical plexus. Thus it may be said that there is an extensive budding of lymphatics from the veins of the neck. Almost all of these lymphatic buds make the deep jugular sac from which vessels arise in three places (1) from the apex, (2) from the anterior curvature and (3) from the stalk of the sac near the valve. These latter vessels are in the main deep lymphatics for the pharynx or superficial vessels which follow the external jugular vein. Occasionally a vessel arises independently from the external jugular vein itself. It may well be brought out here that none of the injections of the deep lymphatics has ever followed the arteries or veins into the cranial cavity.

The origin of the lymphatics of the tongue is peculiar in that its vessels come from two sources. Lying beneath the mandible is the submaxillary plexus, and in the posterior pharynx is the retropharyngeal plexus from the anterior curvature of the sac. The base of the tongue is situated between the two. Into it and into the adjoining part of the pharynx grow ducts from each of these plexuses. At this stage of development the drainage is probably in both directions, but as the size of the embryo increases the more direct retropharyngeal route becomes the chief line,

rather than the roundabout course through the submaxillary plexus. Thus it is clear that the deep lymphatics for the head and neck come from the sac stalk and its extension the anterior curvature. A part of them however, namely the submaxillary group come from the cervical plexus.

To sum up the relations of the superficial lymphatic vessels to the jugular lymph sac; three groups of vessels arise from the apex of the sac and therefore drain into the gland of the posterior triangle of the neck, the suprascapular vessels, the occipital vessels and the superficial cervical plexus. This latter extensive plexus has a double origin coming from the sac stalk as well or even by a variation directly from the vein. The stage of 5 to 6 mm. represents the time when all of the primary superficial plexuses have been formed and have anastomosed with others in the skin so that there may be said to be one continuous plexus of lymphatic capillaries which covers the body. This plexus will become the deep subcutaneous plexus of lymphatic ducts. The development of valves in this plexus which now begins makes it impossible to obtain such extensive injections as can be made in embryos 5 to 6 cm. long but the development of the valves tends again to bring out the primary plexuses which were lost by the development of the anastomoses.

From the anterior curvature of the sac and in part from the stalk of the sac develop the vessels for the pharynx and nose. These together with the submaxillary vessels from the cervical plexus represent the deep lymphatics for the head and neck.

All injections of later stages bring out the fact that the jugular lymph sac develops into two lymph glands and the deep jugular lymph trunk. In figure 4 is shown an injection of the deep lymphatics in the neck of a pig 7.5 cm. long. It shows particularly well the position of the apex of the sac which is now a gland in the posterior triangle of the neck, between the sterno-cleido-mastoid and the trapezius muscles. The anterior curvature of the sac with some of the pharyngeal vessels lies under the sterno-cleido-mastoid muscle. It is also a lymph gland. The sac stalk which is joined by the duct from the apex shows where it emerges from beneath the muscle. On the surface of the sterno-cleido-

mastoid muscle and along its ventral border is the superficial cervical plexus. This is now one large gland. Its efferent vessels are the group of ducts to the apex of the sac and a large group to the sac stalk not injected in this specimen. They show well in other specimens connecting the cervical plexus with the stalk of the sac. They follow the external jugular vein and join the sac near the valve into the vein. The specimen shows some of the afferent vessels of the cervical plexus. Along two of the groups of afferent vessels namely the submaxillary vessels and along the facial vessels are developing lymph glands. This figure may well be compared with the injection of the lymph glands in the neck of a new born given as fig. 30 by Bartels in *Das Lymphgefäßsystem*, in Bardeleben's *Handbuch der Anatomie des Menschen*, p. 103, 1909.

Injections of pigs 7 cm. long show the same structural lines in the lymphatics as the pig measuring 5 cm., with a few modifications. The relations of the jugular sacs are practically the same. One thing must, however, be noted, that the size of the sac has not changed much from that of the 5 cm. pig. Consequently, the relative size of the sac being decreased, it does not occupy as extensive an area in the neck. The anterior curvature instead of being placed below the basi-sphenoid is below the atlas, and the apex lies in the plane between the third and fourth vertebrae instead of extending back to the fifth or sixth.

Another important morphological change is in the marked development of the lymph glands. The region of the sac between the anterior curvature and the apex having probably lost its function, more or less, as a line of drainage, has become considerably reduced in size, with three or four distinct constrictions. The beginning of this change was seen in the pig 5 cm. long.

The third marked change in the sac is the shifting of the cross connection, which in earlier stages passes directly from the apex to the stalk. Instead of connecting by a short vessel with the stalk it passes parallel to it, a connection being finally established near the point where the stalk connects with the vein.

In embryos 8.5 cm. long the sac stalk passes forward external to the internal jugular vein to terminate in a single large node

posterior to the pharynx. From this node lymph vessels radiate to the deep structures of the head outside of the cranial cavity. A single large duct extends back to the apical node lying ventral to the trapezius muscle in the posterior triangle of the neck. This duct is plainly the atrophied remnant of the portion of the lymph sac lying between the apex and the anterior curvature. The cross connection between the apex and stalk lies along the transverse cervical vein and joins the sac stalk near the valve into the veins as in the embryo 7 cm. long. The cervical plexus, consisting practically of large nodes, lies partly in front of the sterno-cleido-mastoid muscle and partly overlaps its anterior border. It is covered superficially by the panniculus carnosus muscle. There are two or three large nodes in front of the ear. Eight or ten ducts lead from the superficial cervical lymph nodes over the sterno-cleido-mastoid muscle, and pass to the primary apical node of the sac. Two or three vessels extend around the posterior margin of the masseter muscle, to the submaxillary lymph glands. In the injected specimens of this stage there is apparently only a single large duct from the cervical plexus along the external jugular vein to the root of the neck. Probably there are more which the injection did not reach. The retropharyngeal and part of the submaxillary vessels drain through the anterior curvature of the sac and the sac stalk. The superficial vessels of part of the head, the face, neck, thorax and arm drain through the superficial cervical glands either to the glands of the posterior triangle or directly to the sac stalk through the external jugular lymph trunks. The occipital and suprascapular vessels drain through the gland of the posterior triangle of the neck.

Dissections of the adult pig show that the gland which develops from the apex of the sac remains as a single gland in the posterior triangle of the neck. In one specimen it measured 2 by 3 cm. long. The gland from the anterior curvature of the sac also remains a single gland. It is not as large as the apical gland measuring 1 by 2 cm. It lies on the lateral surface of the internal jugular vein just dorsal to the pharynx. No other large gland is to be found along the internal jugular vein but there are a few small ones. It is clear that the gland of the posterior triangle

of the neck and the pharyngeal gland of the pig are represented by groups of glands in the human being. The glands of the posterior triangle of the neck in a new born are well shown in the figure by Bartels, quoted above.

In the adult pig the superficial cervical plexus becomes a group of from twelve to eighteen glands lying along the external surface of the sterno-cleido-mastoid muscle. They are of all sizes varying from 1 by 2 cm. long to one very large gland measuring 2 by 3 cm. just behind the ear near the origin of the sterno-cleido-mastoid muscle. This group of superficial glands clearly come from the superficial cervical plexus and the vessels which grow from it. The efferent vessels from the superficial glands show well in Bartel's figure, that is those that run to the glands in the posterior triangle. The development of the superficial plexus leads one to expect that other efferent vessels follow the external jugular vein.

From this study it is possible to obtain a clear idea of all the lymph channels in the neck of the pig. From the apex of the jugular lymph sac develops a large node in the posterior triangle of the neck which receives the occipital, the suprascapular and the superficial cervical lymphatics, that is to say practically all of the superficial lymphatics for the anterior part of the body. The rest of the sac becomes the deep jugular lymph trunks and lymph glands of which the most cerebral one is by far the largest. The deep jugular glands drain the pharynx and Eustachian tube, the nose and in part the vessels of the tongue and lower jaw. The superficial cervical group of glands has the most complicated development. This group comes not only from the apex of the sac around the dorsal surface of the sterno-cleido-mastoid muscle but from the sac stalk or deep jugular trunk by vessels which follow the external jugular vein. It may also have a direct vessel from the veins. The superficial cervical plexus drains the posterior auricular region, the temporal, facial and cervical regions as well as the thoracic wall and the skin of the arm. The deep or axillary lymphatics of the arm have not yet been followed in the pig.

One of the most important points in this study is that there are primary plexuses of lymphatics for each region, that these primary

plexuses are at first distinct connecting with each other only through the lymph sac which makes their common point of origin. Soon anastomoses between these primary plexuses connect them so abundantly that the entire surface of the body is covered with a primary and continuous plexus of lymphatics all of which can be injected from any one point. The subsequent development of valves follows the pattern of the original primary ducts as can be seen in figure 505 in the *Handbuch der Entwicklungsgeschichte des Menschen*, Keibel and Mall, p. 708, 1911, to such an extent that the primary lines of growth can be made out in injections. The study shows exactly what is meant by saying that the jugular lymph sac becomes transformed into lymph glands. The glands which arise from the sac itself we call primary lymph glands, those which develop on the vessels which grow from the sac may be called secondary and tertiary glands. The three kinds of glands are all shown in figure 4. Lymph glands develop at the points from which the peripheral lymphatics radiate out.

ON THE CHEMICAL NATURE OF CERTAIN GRANULES IN THE INTERSTITIAL CELLS OF THE TESTIS

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SIX FIGURES

In the course of a previous communication the writer¹ called attention to the existence of certain granules in the interstitial cells of the testes of various mammals. The present paper gives the results of an attempt to control the previous observations, and also to obtain some insight into the chemical nature of these bodies.

The granules were detected first in the cat's testis fixed in formalin, and I shall begin the present account by a brief description of the appearances noted in such material. In paraffin sections the granules may be demonstrated by a number of staining methods, of which Reinke's neutral gentian as employed by Bensley² is excellent. Here the cytoplasmic framework of the cells is stained by the orange G, while the granules take the violet dye (fig. 1), as does also the chromatin of the nucleus. The cytoplasm is extensively vacuolated owing, for the most part, to the solution of large quantities of lipoids by the reagents employed in imbedding. The violet-colored granules are fairly spherical in contour, and vary as to size. They are sprinkled here and there in the cell-body, but are most numerous in the peripheral portion of the

¹ R. H. Whitehead. Studies of the interstitial cells of the testis. *Histology. Anat. Rec. of Amer. Jour. Anat.*, vol. 7, no. 4, 1908. I have remarked elsewhere that this paper is catalogued "in the German literature" under a misleading title. Dr. P. Mayer informs me that it is cited correctly in *Zool. Jahresb. f.* 1908. My statement was too broad, and I take the first opportunity to correct it.

² R. R. Bensley. Studies on the pancreas of the guinea pig. *Amer. Jour. Anat.*, vol. 12, 1911.

cell. However, they may be located in any portion of the cell-body; often some are found in the vicinity of the nucleus, occasionally in apparent contact with the nuclear membrane. It can be determined that they lie in vacuoles, though some of the smallest granules appear to be located at the nodal points of cytoplasmic threads. It would seem that the number of the granules varies with different individuals. Thus in one cat there were few cells observed that contained more than two or three granules; in this animal the number and size of the cells also seemed decidedly below the average.

A very clear picture was obtained with Wright's blood stain. The sections were stained twenty-four hours in a dilute solution (one part of dye to forty of distilled water), the excess of dye was washed out with distilled water, and the sections were then dehydrated in acetone, cleared in xylol, and mounted in neutral balsam. The granules were stained red, the chromatin took the methylene blue, and the general cytoplasm was stained blue or pinkish blue (fig. 2). Sections of this material stained with Altmann's solution of acid fuchsin gave essentially the same appearances so far as the granules were concerned; but in this case the picture was not so clear owing to the fact that both the cytoplasm and the granules were stained by the acid fuchsin; the granules, however, were a much deeper red. I would call especial attention to the fact that in none of the preparations mentioned above were any mitochondrial structures demonstrated in the seminal epithelium.

Iron haematoxylin gave a somewhat different picture (fig. 3). Here the spongioplasmic framework was well stained in many cells, so that it sometimes presented the appearance of threads of small granules (mitochondria). In such cases granules might be present in somewhat confusing number; but the distinction between the two sorts could usually be made owing to the fact that the granules were larger than the spongioplasmic structures, were more regularly spherical, and were contained in vacuoles, whose walls were furnished by trabeculae of the spongioplasm. In these preparations mitochondrial structures were seen also in the seminal epithelium, threads of bacilli-like grains in the Sertoli cells being brought out with especial distinctness.

In material fixed in absolute alcohol the granules, in spite of the distortion of the cells, were still quite evident and apparently undiminished in number.

In the previous communication it was stated that the granules could not be stained in material fixed by solutions which contained potassium bichromate in large proportion. This statement was based on the negative results obtained with pieces of cat's testis fixed in several fluids which contained that salt in considerable amount. I am unable to say just where the error came in, but the statement was certainly erroneous. Hanes³ has demonstrated the granules in pig's testis fixed in Zenker's fluid, and I have myself repeatedly observed them in this material fixed by Zenker. I have investigated the point anew in the cat's testis as well as the pig's, and find that the granules, while present, are more or less disfigured: they are apt to lose their regularly spherical contour and appear as irregular lumps, or as spherules with darkly stained periphery and lighter center. This disfiguration, however, is possibly due to the acetic acid contained in the Zenker's fluid.

From the above we may conclude that the granules described in formalin and absolute alcohol material are proteid in nature. They may be stained with either basic (gentian violet) or acid (acid fuchsin) dyes; but when given an equal opportunity at the methylene blue and the eosin in Wright's stain take the eosin.

In the course of a recent study of the lipoids of these cells⁴ in the cat it was noticed that along with the globules of lipoids the interstitial cells frequently contained granules with an affinity for Sudan III which, in respect of size and position, were indistinguishable from the granules just described. The suggestion presented itself that the granules really consist of a combination of proteid and fatty material, and that in paraffin sections of material fixed in formalin or absolute alcohol the fatty constituent had been dissolved out, leaving only the proteid. It seemed highly improbable that any fatty matter would remain in sections

³ F. M. Hanes. The relation of the interstitial cells of Leydig to the production of an internal secretion of the mammalian testis. *Jour. Exp. Med.*, March, 1911.

⁴ R. H. Whitehead. A microchemical study of the fatty bodies of the interstitial cells of the testis. *Anat. Rec.*, vol. 6, 1912.

of material which had been subjected to this treatment, but inasmuch as Fauré-Fremiet, Mayer and Shaeffer⁵ have reported that some combinations of proteid and fat are precipitated by formalin, the point was tested by staining paraffin section of formalin material with Sudan. The results were always negative; even the strong solution of Sudan made by the addition of caustic soda, according to Herxheimer's method with scharlach, left the granules unstained.

Various attempts were made to verify the hypothesis that the granules consist of a combination of proteid with fatty material by staining methods intended to demonstrate the granules and the lipid globules in different colors in the same cell, but as none of these yielded decisive results, I may omit a detailed description of them, and state the results of two which were, at least, strongly suggestive. Pieces of formalin material were treated for three days with Flemming's fluid, imbedded in paraffin, sectioned, and the sections stained with neutral gentian and mounted in xylol balsam. In such sections the lipid globules as well as the granules were stained by the gentian, but the violet color rapidly faded out of the globules leaving the granules stained violet. Again, in sections of material fixed by Ciaccio's method and stained with Sudan and iron haematoxylin lipid globules were stained red, while here and there among them were small granules stained by the haematoxylin. It did not seem possible, however, to identify these latter absolutely with the granules in question, because it is known that some lipoids after treatment with potassium bichromate give a lake with haematoxylin; and so these haematoxylin-stained grains may have been simply such lipoids without any proteid admixture. Thus the large amount of lipoids in the cat's testis introduced so much confusion into the picture that it did not seem possible to get decisive results from this method of investigation. Accordingly recourse was had to the pig's testis, as a previous study⁶ of it had shown almost entire

⁵ Fauré-Fremiet, Mayer et Shaeffer. Sur la microchemie des corps gras. Arch. d'Anat. Microsc., T. 12, 1910.

⁶ R. H. Whitehead. Studies of the interstitial cells of the testis. Histology. Anat. Rec. of Amer. Jour. Anat., vol. 7, no. 4, 1908.

absence of lipid material so far as the interstitial cells were concerned.

In the pig the general histological characters of the interstitial cells are essentially the same as in the cat; there are, however, some differences which require to be mentioned. The lipid globules which are so abundant in the cat are virtually absent; consequently the large vacuoles left in the cytoplasm by the solution of these bodies are also absent, and the spongionoplasmic framework is much tighter. There is, however, an abundance of small vacuoles towards the periphery of the cells. In sections of formalin material stained by neutral gentian the appearances presented by the granules is about the same as in the cat, except that they are, perhaps, more variable as to size. In preparations stained by iron haematoxylin or by Altmann's acid fuchsin or by Benda's method (after fixation in Flemming's fluid) and in which differentiation has not been carried very far, there is often an embarrassment of granules, and the entire cell-body may appear stuffed with them. A little study shows, however, that in the vicinity of the nucleus small grains predominate, while towards the periphery of the cell larger ones become more numerous; moreover, the small granules often appear arranged in threads. If the differentiation has been well carried out, until the structure of the nucleus is well shown, the small grains give up the stain and the picture does not differ from that exhibited by the preparations stained with neutral gentian, i.e., a number of granules lying in small vacuoles and most numerous at the periphery of the cell (fig. 4). I take it that the small granules arranged in threads are the mitochondrial structures of the cells, and that the larger ones in vacuoles are the granules under discussion.

This material seemed ideal for applying the test of direct staining of the granules with a specific fat dye such as Sudan III, as there should be absence of the confusion encountered in the cat's testis due to the presence of many globules of lipoids. Thin frozen sections were stained for half an hour in the incubator at 37° C. with a saturated solution of Sudan III in 70 per cent alcohol, but on examination proved practically negative. Fatty material within the seminiferous tubules was demonstrated in large amount

but the interstitial cells contained no red-staining material. Thinking that the failure to stain might be due to the small size of the granules, recourse was had to the strong solution recommended by Herxheimer for scharlach (absolute alcohol 70 cc., 10 per cent solution of caustic soda 20 cc., distilled water 10 cc., Sudan to saturation) with the result that sections stained ten or fifteen minutes at room temperature offered a very satisfactory demonstration of the granules in red. Some of the cells presented a quite exact reproduction in red of the picture of the granules as seen in paraffin sections stained by the other methods. They were situated in small vacuoles between the trabeculae of the spongioplasm, and were most numerous at the periphery of the cells (fig. 5). It is to be noted, however, that many of the granules appeared smaller than in the other preparations; and in many of the cells this small size of the red granules was quite pronounced, as if only a localized portion of their substance had been stained by the Sudan (fig. 6). Thus there was obtained direct proof that the granules contained fatty material in addition to the proteid constituent demonstrated by the other methods.

It remained to determine whether or not these granules should be classed with the lipochromes, as fatty pigment has been found in the interstitial cells of several mammals. According to Sehrt,⁷ who has made an extensive study of this pigment in the human testis, it is stained by Sudan even in sections of material that has been imbedded in paraffin. In the case of the pig and the cat the granules were colorless in frozen sections, and it was not possible to stain them with Sudan in paraffin sections of formalin material. It seems certain, therefore, that the granules are not lipochrome.

From this study I conclude that the granules in the interstitial cells of the pig, and probably also of the cat, consist of a combination, either physical or loosely chemical, of proteid with fatty material. It also shows that the pig's interstitial cells, which contain no individual globules of lipoids, are no exception to the

⁷ Sehrt. Zur Kenntniss der fetthaltigen Pigmente. Virchow's Arch., Bd. 177, 1904.

general rule that these cells, aside from any other function that may be ascribed to them, serve as a storehouse for fatty material.

Finally, one may ask if these observations throw any light upon the histological nature of the granules. Are they derived from the chromatin of the nucleus, i.e., are they chromidial in nature? In favor of a nuclear origin is the fact that while the majority of the granules lie in the peripheral portion of the cell, it is common to find some in the neighborhood of the nucleus; in fact, they may be found lying against the nuclear membrane. Moreover, with most staining methods the granules and the chromatin are stained by the same dye. Such observations are, of course, merely suggestive. On the other hand, when the granules and the chromatin were given an equal opportunity at an acid and a basic dye without any more differentiation than was obtained by washing with distilled water, the chromatin took the basic, while the granules preferred the acid dye (fig. 2). So that, if one assumed that the granules are derived from the nucleus, he would also have to assume that they undergo a change in chemical nature as soon as they escape from the nucleus. Somewhat more direct seems the evidence in favor of their derivation from the cytomicrosomes—Altmann's granules, mitochondria, or whatever name shall be given to the small granules located in the trabeculae of the spongioplasm. As we have seen, it is quite possible to stain these at the same time with the granules. Apparently all that is needed to convert them into the granules is the incorporation of fatty material and increase in size; there would thus be produced the picture of granules lying in small vacuoles of the spongioplasm.

Another question which presents itself is, are these granules mitochondria? If Benda's dictum is correct, that his staining method for mitochondria is specific and that all cytoplasmic structures which stain by this method are mitochondria, then these granules are undoubtedly mitochondria. It may be pointed out further that Fauré-Fremiet,⁸ Regaud and others have presented evidence—evidence based for the most part upon deduc-

⁸ Fauré-Fremiet, Mayer et Shaeffer. *Sur la microchimie des corps gras*. Arch. d'Anat. Microsc., T. 12, 1910.

tions from the chemical processes believed to occur during fixing and staining—to show that mitochondria chemically considered consist of a combination of proteid with fatty material, which as we have seen, is also the constitution of the granules described in this paper. On the other hand, so far as I am aware, undoubted mitochondria, such as those of the sex cells, have not been stained by a specific fat stain. However that may be, it does not seem possible that the mitochondria of the sex cells of the cat and pig can be identical with the granules in the interstitial cells; for in my paraffin sections of formalin material stained by neutral gentian or Wright's blood stain the granules of the interstitial cells were demonstrated very clearly, while in the seminal epithelium absolutely no granules were stained. So that, for the present, one may be permitted to believe with Heidenhain,⁹ that while Benda has probably brought to light new structures in the case of the mitochondria of sex cells, it is carrying his results too far to identify mitochondria with all cytoplasmic structures which can be stained by his method.

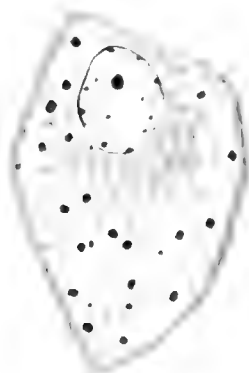
PLATE 1

EXPLANATION OF FIGURES

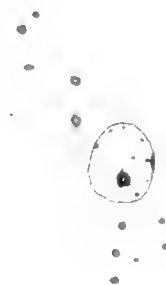
All the cells were outlined with the camera lucida. Reichert, obj. $\frac{1}{2}$.

- 1 Interstitial cell of cat; neutral gentian. Formalin fixation.
- 2 Interstitial cell of cat; Wright's blood stain. Formalin fixation.
- 3 Interstitial cell of cat; iron haematoxylin. Formalin fixation.
- 4 Interstitial cell of pig; iron haematoxylin. Formalin fixation.
- 5 Interstitial cell of pig; Sudan III. Frozen section.
- 6 Interstitial cell of pig; Sudan III. Frozen section.

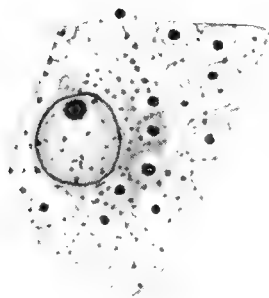
⁹ M. Heidenhain. Plasma und Zelle, 1907.



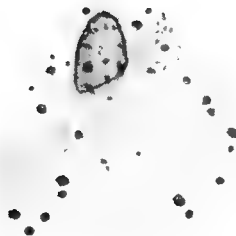
1



2



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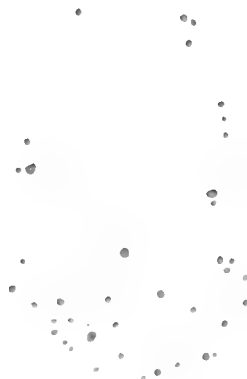


4



5

PHOTO-CHROMOTYPE, PHILA. PA.



6

A COMPARATIVE STUDY OF THE THREE PRINCIPAL REGIONS OF THE SPINAL CORD IN A SERIES OF MAMMALS

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TWENTY-FIVE FIGURES

This paper presents some observations based on the study of transverse sections of the three principal regions (intumescencia cervicalis, intumescencia lumbalis, and middle of pars thoracalis) of the spinal cord in a series of twenty-four mammals. An outline drawing of a transverse section of the cords in each region is included together with tables of measurements showing variations in dimension and structure of the cords in the different mammals.

MATERIAL AND METHODS

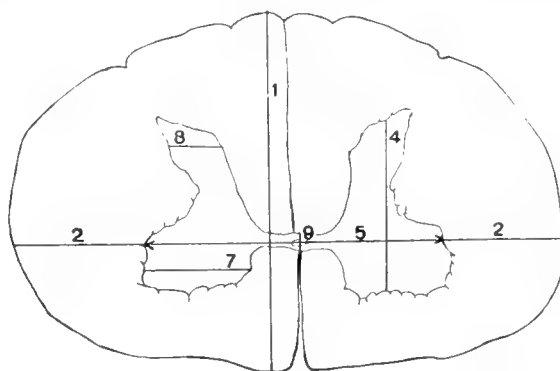
In most cases one representative only of a species of mammal was studied. Hence all error due to individual variation is included in this work. An average taken from the study of a large number of individuals of each species would obviously be of greater value. In so far as was possible to ascertain, all animals used were normal. A few of the specimens were kindly furnished by Professor Hardesty with the sections ready for study. The method by which they had been prepared, and which was used for the remaining specimens of the list is, very briefly, as follows.

Most of the spinal cords were removed entire from the vertebral columns as soon after the death of the animal as possible and placed in a 10 per cent solution of formalin. After fixation a segment through each of the enlargements (cervical and lumbar) and the smallest portion of the thoracic region was cut from the cord. The blocks which were taken from the sixth cervical, the eighth thoracic, and the third lumbar segments were mordanted five to ten days in Muller's fluid, embedded in celloidin, and sections cut at 30 micra. The sections were stained by Pal's modification of the Weigert method for medullated nerve fibers.

Drawings. The figures were made in outline by means of the Edinger projection apparatus set for a magnification of eight diameters. They are not intended to present detail of structure but are of value in comparing the form of the several components of the different cords. What little detail is given, however, was obtained with the compound microscope (Leitz, Objective 2, Ocular 3). In the case of the lumbar regions of the ox and of the horse, one side of the largest segment of which was slightly injured in removing the cord, bilateral symmetry was assumed and the injured side was reconstructed from the intact half. With the exception of the lumbar region of the elephant which I was unable to obtain, the three drawings from the cord of each animal in the series is complete.

Tables. Data obtained for use in making the numerous general comparisons of the structures of the spinal cord is accumulated in the following tables with the animals arranged according to body weight. Table 1 gives a complete list of the animals with body weights. The underscored figures are true body weights of the animals, the others approximate. Tables 2, 3, and 4 record for each region certain measurements taken according to the scheme shown in text figure A the lines in which indicate the extent and direction of each measurement. The numbers of the lines in this figure correspond to like numbers in the tables. In all cases measurements of each half of the cord were taken and the average recorded after dividing by eight, the magnification represented by the drawings. Table 5 is constructed from

the data in tables 2, 3, and 4 and is described in the discussion of it. Table 6 records the exact areas of the several components of the cord as obtained by tracings of the drawings with the Amsler planimeter. Separate readings were taken for the area of the grey figure, the area of the dorsal funiculus, and the total area. Other measurements were obtained by computing differences. Each tracing with the planimeter was taken three times and the average computed to minimize mechanical errors. The



Text figure A. Outline of a section of the spinal cord with lines, numbered, indicating the extent and direction of each of the measurements taken of the different sections of the various spinal cords dealt with. These measurements are recorded in tables 2, 3, and 4. The numbers of the lines correspond to like numbers in the columns of the tables: 1, dorso-ventral diameter; 2, lateral diameter; 4, thickness of grey figure; 5, width of grey figure; 7, width of ventral horn; 8, width of dorsal horn; 9, width of grey commissure.

average of the three readings, which were in terms of square centimeters and fractions of square centimeters including vernier readings, were reduced by sixty-four, the square of the magnification. The actual areas of the specimens thus obtained are here recorded in terms of square millimeters. For the sake of convenience, I have selected certain data from table 6 and compiled the tables of ratios, 7, 8a, and 8b.

TABLE 1

Table 1 gives the mammals used arranged in series in order of their absolute and approximate body weights. In case of certain of the specimens, obtained some years ago by Professor Hardesty and some obtained since, the body weight was not determined and here had to be estimated. The underscored weights are absolute weights, the others are approximate.

	KILOS	POUNDS
Elephant (<i>Elephas indicus</i>).....	3,628.736	8000
Horse (<i>Equus caballus</i>).....	566.990	1250
Ox (<i>Bos taurus</i>).....	430.910	950
Brown bear (<i>Ursus americanus</i>).....	136.077	300
Hog (<i>Sus scrofa</i>).....	68.038	150
Man (<i>Homo sapiens</i>).....	72.574	160
Sheep (<i>Ovis aries</i>).....	34.019	75
Kangaroo (<i>Macropus giganteus</i>).....		
Black crested ape (<i>Macacus inuus</i>).....	5.216	
Dog (<i>Canis familiaris</i>).....	5.000	
Gray fox (<i>Urocyon cinereo-argentatus</i>)....	3.940	
Lynx (<i>Felis rufus</i>).....	3.050	
Raccoon (<i>Procyon loctor</i>).....		
Rhesus monkey (<i>Macacus rhesus</i>).....	2.720	
Opossum (<i>Didelphys Virginiana</i>).....		
Cat (<i>Felis domestica</i>).....	2.300	
Spider monkey (<i>Ateles paniscus</i>).....	1.650	
Java monkey (<i>Semnopithecus maurus</i>)....		
Rabbit (<i>Lepus cuniculus</i>).....	1.560	
Agouti (<i>Dasyprocta agouti</i>).....	1.350	
Guinea-pig (<i>Cavia copaya</i>).....	.610	
Gray rat (<i>Mus Norvegicus</i>).....	.500	
White mouse (<i>Mus musculus, albus</i>).....	.027	
Bat (<i>Nyctinomus brasiliensis nasutus</i>)....	.016	

TABLE 2

Measurements of cervical region in millimeters. Table 2 includes measurements of transverse sections from the cervical enlargement. The numbers in the heads of the columns correspond to the lines of measurement so numbered in text figure A, and the figures record actual size of the sections.

ANIMAL	1. DORSO-VENTRAL DIAMETER	2. LATERAL DIAMETER	3. MEAN DIAMETER	4. THICKNESS GREY FIGURE	5. WIDTH GREY FIGURE	6. MEAN GREY FIGURE	7. WIDTH VENTRAL HORN	8. WIDTH DORSAL HORN	9. WIDTH GREY COMMISSURE
Elephant.....	17.06	29.78	23.42	8.21	13.68	10.94	6.00	4.43	1.12
Horse.....	13.43	23.28	18.35	7.28	8.81	8.04	4.00	2.50	0.68
Ox.....	11.15	17.85	14.50	5.56	7.18	6.37	3.84	2.60	0.56
Bear.....	11.06	13.65	12.35	5.87	7.75	6.81	3.37	1.60	1.12
Hog.....	9.71	11.00	10.35	5.18	4.00	4.59	2.62	1.18	0.43
Man.....	8.93	13.81	11.37	4.25	7.12	5.68	2.75	1.37	0.62
Sheep.....	8.92	11.18	10.05	4.87	5.12	4.99	2.78	1.85	0.75
Kangaroo.....	7.50	8.84	8.17	3.58	3.12	3.35	1.43	1.75	0.87
Ape.....	6.60	7.71	7.15	3.62	3.62	3.62	1.82	1.12	0.62
Dog.....	5.21	7.81	6.51	3.06	4.87	3.96	2.10	1.12	0.31
Fox.....	7.73	7.78	7.57	3.87	3.46	3.66	1.81	1.03	0.62
Lynx.....	9.43	10.71	10.07	4.98	5.87	5.42	2.62	1.60	0.50
Raccoon.....	7.37	7.78	7.57	4.31	3.65	3.98	1.50	1.28	0.56
Rhesus monkey.....	4.81	6.50	5.65	3.00	2.93	2.96	1.75	1.25	0.56
Opossum.....	4.62	6.15	5.38	2.62	3.06	2.84	1.43	1.21	0.37
Cat.....	5.90	7.06	6.48	3.43	3.37	3.40	1.75	1.31	0.50
Spider monkey.....	5.68	7.00	6.34	3.70	4.12	3.91	1.84	1.59	0.62
Java monkey.....	5.68	6.31	5.99	3.75	3.25	3.50	1.50	1.06	1.12
Rabbit.....	4.60	5.59	5.09	2.89	4.10	3.39	1.43	1.01	1.12
Agouti.....	6.71	6.43	6.57	3.59	3.25	3.42	1.50	1.10	0.37
Guinea-pig.....	2.87	4.06	3.46	1.93	2.18	2.05	1.12	0.81	0.43
Rat.....	2.90	4.50	3.70	1.87	2.84	2.35	1.18	0.87	0.43
Mouse.....	1.48	2.56	2.02	1.09	1.68	1.38	0.78	0.75	0.25
Bat.....	1.31	2.93	2.12	1.25	1.93	1.59	0.93	0.87	0.15

TABLE 3

Measurements of thoracic region in millimeters. Table 3 records measurements of transverse sections from the thoracic region, taken as those recorded in table 2.

ANIMAL	1. DORSO-VENTRAL DIAMETER	2. LATERAL DIAMETER	3. MEAN DIAMETER	4. THICKNESS GREY FIGURE	5. WIDTH GREY FIGURE	6. MEAN GREY FIGURE	7. WIDTH VENTRAL HORN	8. WIDTH LORSAL HORN	9. WIDTH GREY COMMISSURE.
Elephant.....	14.65	18.56	16.60	5.71	4.68	5.19	1.10	1.75	0.75
Horse.....	12.25	13.25	12.75	5.50	3.12	3.81	1.18	1.21	0.68
Ox.....	9.87	11.18	10.52	3.85	3.00	3.42	1.01	1.14	1.65
Bear.....	6.87	8.12	7.49	2.31	2.81	2.56	0.84	0.87	1.18
Hog.....	7.00	7.56	7.28	3.12	1.68	2.40	0.62	0.53	1.12
Man.....	7.31	8.50	7.90	2.68	2.43	2.55	0.93	0.50	0.37
Sheep.....	6.31	6.62	6.46	3.12	1.87	2.49	0.56	0.62	1.06
Kangaroo.....	6.81	7.15	6.98	2.87	1.56	2.21	0.50	0.56	1.31
Ape.....	4.15	4.68	4.41	1.75	1.18	1.46	0.32	0.87	0.56
Dog.....	3.76	4.59	4.17	1.62	2.09	1.85	0.65	0.53	0.56
Fox.....	5.15	5.06	5.10	2.18	1.25	1.71	0.46	0.26	0.23
Lynx.....	5.35	6.50	5.92	2.12	1.87	1.99	0.64	0.71	1.18
Raccoon.....	4.62	4.87	4.74	2.03	1.15	1.59	0.40	0.53	1.00
Rhesus monkey.....	3.37	4.93	4.15	1.37	1.43	1.40	0.40	0.62	0.93
Opossum.....	3.60	3.93	3.76	1.75	1.18	1.46	0.16	0.43	0.56
Cat.....	4.18	4.86	4.52	1.78	1.75	1.76	0.50	0.62	0.56
Spider monkey.....	4.12	4.12	4.12	2.31	1.18	1.74	0.28	0.43	1.12
Java monkey.....	3.71	3.71	3.71	1.96	0.68	1.32	0.37	0.25	1.37
Rabbit.....	3.84	4.53	4.18	1.93	1.62	1.77	0.64	0.56	0.75
Agouti.....	5.12	5.37	5.24	2.31	1.18	1.74	0.50	0.56	0.43
Guinea-pig.....	2.78	2.87	2.82	1.78	1.12	1.45	0.45	0.65	0.31
Rat.....	2.81	2.62	2.71	1.75	0.87	1.31	0.25	0.34	0.37
Mouse.....	1.31	1.56	1.43	0.84	0.68	0.76	0.28	0.43	0.37
Bat.....	1.28	1.59	1.43	1.00	1.25	1.12	0.31	0.71	0.25

TABLE 4

Measurements of lumbar region in millimeters. Table 4 records measurements of transverse sections from the lumbar region, taken as those recorded in table 2.

ANIMAL	1. DORSO-VENTRAL DIAMETER	2. LATERAL DIAMETER	3. MEAN DIAMETER	4. THICKNESS GREY FIGURE	5. WIDTH GREY FIGURE	6. MEAN GREY FIGURE	7. WIDTH VENTRAL HORN	8. WIDTH DORSAL HORN	9. WIDTH GREY COMMISSURE
Horse.....	13.12	21.71	17.41	7.51	10.90	9.21	5.31	3.71	0.50
Ox.....	9.18	16.00	12.59	5.84	9.00	7.42	3.75	3.37	0.50
Bear.....	8.93	10.46	9.69	5.00	5.87	5.43	2.83	1.65	0.75
Hog.....	7.56	9.56	8.56	3.68	4.75	4.21	1.62	1.40	0.68
Man.....	8.53	9.50	9.01	4.93	5.37	5.15	2.50	1.81	0.62
Sheep.....	7.29	10.31	8.80	4.37	5.50	4.93	2.71	2.10	0.62
Kangaroo.....	8.45	10.12	9.28	4.65	4.25	4.45	2.65	2.00	0.56
Ape.....	4.92	6.28	5.60	3.00	4.25	3.67	1.90	1.43	0.37
Dog.....	4.75	6.34	5.54	3.21	4.06	3.63	2.12	1.32	0.50
Fox.....	6.31	7.12	6.71	3.81	3.81	3.81	1.68	1.18	0.56
Lynx.....	7.81	9.65	8.73	5.12	5.78	5.45	2.45	1.67	0.33
Raccoon.....	6.21	6.81	6.51	3.71	4.50	4.10	2.09	1.43	0.71
Rhesus monkey.....	4.50	5.81	5.15	3.06	3.37	3.21	1.65	1.25	0.50
Opossum.....	4.51	5.56	5.03	2.93	3.12	3.02	1.76	1.46	0.71
Cat.....	5.31	6.43	5.87	3.43	3.50	3.46	1.75	1.43	0.37
Spider monkey.....	5.46	5.56	5.51	3.50	3.87	3.68	1.93	1.78	0.62
Java monkey.....	4.75	5.75	5.25	3.62	4.06	3.84	1.87	1.37	0.87
Rabbit.....	4.02	6.14	5.08	2.65	4.12	3.38	1.89	1.25	0.56
Agouti.....	5.90	6.87	6.38	3.75	5.06	4.40	2.37	1.75	0.75
Guinea-pig.....	2.85	3.87	3.36	1.96	2.37	2.16	1.16	1.15	0.31
Rat.....	2.59	4.31	3.45	1.71	2.87	2.29	1.31	0.87	0.35
Mouse.....	1.45	2.12	1.78	1.03	1.37	1.20	0.65	0.60	0.35
Bat.....	1.31	2.25	1.78	1.12	1.56	1.34	0.75	0.81	0.25

OBSERVATIONS

Common structures

In glancing over the drawings, one of the first things which attracts attention is the marked similarity of form throughout the series. Each cord is possessed of bilateral symmetry and presents a grey figure in the shape of the letter *H*, at times considerably modified. Each half of the grey figure of the cord has the ventral and dorsal horn, the gelatinous substance of Rolando being found at the apex of the latter. The two sides of the grey figures are connected by a commissure in which is a central canal surrounded by a homogeneous substance (central gelatinous substance) similar in structure to the gelatinous substance of Rolando. The white substance surrounding the grey is divided into a dorsal and a ventro-lateral funiculus by the more or less well marked dorso-lateral sulcus, the line of ingrowth of the dorsal root. The dorso-intermediate sulcus provides an interesting comparison which will be mentioned when the dorsal funiculus is considered. Each section shows a ventral median fissure but not all, in Weigert sections at least, presents a dorsal median septum. The reticular formation, present in all, varies considerably in amount as the drawings show.

Diameters

Text-books on neurology state that the human spinal cord in each region has a lateral diameter greater than the dorso-ventral. Authorities agree that the greatest difference in these diameters, or in other words, the portion of the cord most flattened dorso-ventrally, is the cervical region. Cunningham '09) states that the lumbar region is more nearly circular than the thoracic. Piersol ('10) on the other hand, gives the lumbar region as being more flattened than the thoracic. The measurements for man here given agree with those of Cunningham. However measurements taken from several other specimens in this laboratory agree more nearly with Piersol.

From table 5, which records in the form of a ratio the relative amount of flattening in each region, it is seen that the bat has a

cord more flattened in its cervical region than any other animal in the series here studied. This may be accounted for in part by the extensive innervation required by the greatly developed wing musculature.

The thoracic region, in general for all the spinal cords, is flattened dorso-ventrally. In most cases the flattening is much less than in either the cervical or the lumbar region. The cords of the agouti, lynx, and rhesus monkey are peculiar in that they

TABLE 5

Ratio of dorso-ventral to lateral diameter. Table 5 gives the ratios of the dorso-ventral to the lateral diameter obtained by dividing the figures in column 2, tables 2, 3, and 4 by those in column 1 of the same tables. That is to say, the dorso-ventral diameter is to the lateral diameter as 1 is to the figures here recorded. In each column the animals are arranged in the order of the dorso-ventral flattening of the regions of their spinal cords, those showing the greater dorso-ventral flattening being placed first. The ratio for the lumbar region of the elephant is obtained from diameters given by Kopsch, as quoted from Hardesty.

CERVICAL 1	THORACIC 2	LUMBAR 3
Bat.....2.23	Rhesus monkey...1.46	Ox.....1.74
Elephant.....1.74	Elephant.....1.26	Bat.....1.71
Horse.....1.73	Bat.....1.24	Rat.....1.66
Mouse.....1.72	Dog.....1.22	Horse.....1.65
Ox.....1.69	Lynx.....1.21	Rabbit.....1.52
Rat.....1.55	Mouse.....1.19	Elephant.....1.52
Man.....1.54	Bear.....1.18	Mouse.....1.46
Dog.....1.49	Rabbit.....1.17	Sheep.....1.41
Guinea-pig.....1.41	Man.....1.16	Guinea-pig.....1.35
Rhesus monkey.....1.35	Cat.....1.16	Dog.....1.33
Opossum.....1.33	Ox.....1.13	Rhesus monkey.....1.29
Sheep.....1.25	Ape.....1.12	Ape.....1.27
Spider monkey.....1.23	Opossum.....1.09	Hog.....1.26
Bear.....1.23	Hog.....1.08	Lynx.....1.23
Rabbit.....1.21	Horse.....1.08	Opossum.....1.23
Cat.....1.19	Raccoon.....1.05	Cat.....1.21
Kangaroo.....1.17	Kangaroo.....1.04	Java monkey.....1.21
Ape.....1.16	Agouti.....1.04	Kangaroo.....1.19
Lynx.....1.13	Sheep.....1.04	Bear.....1.17
Hog.....1.13	Guinea-pig.....1.01	Agouti.....1.16
Java monkey.....1.11	Spider monkey...1.00	Fox.....1.12
Fox.....1.05	Java monkey....1.00	Man.....1.11
Raccoon.....1.05	Fox.....0.98	Raccoon.....1.09
Agouti.....0.95	Rat.....0.90	Spider monkey.....1.01

possess greater flattening in the thoracic than in the cervical region, the sections of which latter are approximately circular. Man, bear, and rhesus monkey stand alone in having a cord in which the thoracic region possesses more dorso-ventral flattening than does the lumbar region.

In about half the mammals of the series, including man, the cervical region of the cords shows a greater dorso-ventral flattening than the lumbar region. In the other half the order is reversed, the greater flattening occurring not in the cervical but in the lumbar region. Chief among this latter number are the sheep, rabbit, hog and Java monkey which, as will be noted, have relatively a very small dorsal funiculus. While on the other hand the bat, mouse, and man have greatly flattened cervical regions and very large dorsal funiculi (column 4, table 8b). The shape of the cords in the series varies to such an extent in each region that it is difficult to say that any given cord is of a predominately rounded or flattened form throughout its length. However, from data here given we may say the bat and elephant are types of dorso-ventrally flattened cords while the fox possesses a cord of rounded form.

Enlargements and total area •

As is well known the cervical and lumbar enlargements are the result of a response to the increased demand for innervation made by the extremities. Furthermore, four-footed animals with approximately equally developed extremities have the area of the cervical enlargement greater than that of the lumbar, due largely to the fact that the cervical region is concerned with the innervation of the tissues of the thorax in addition to those of the upper extremity as well as to the fact that this region carries all the fibers connecting the regions below it with the brain. The kangaroo, with its very small anterior extremities, has a lumbar enlargement the total area of which in transverse section is 13.99 sq. mm. greater than the total area of the section of its cervical enlargement (column 1, table 6, and fig. 8, *C* and *L*).

Schmidt ('08) states that the *Dipus* (*laculus*), a kangaroo-like rodent, has, notwithstanding its relatively small anterior extremities, a cervical enlargement which exceeds the lumbar. He suggests that the size of the cervical enlargement may be due to the relatively more active anterior extremity.

A very striking illustration of the significance of the enlargements is given by Streeter ('03) in a description of the ostrich cord. The ostrich, according to Streeter, has an insignificant cervical enlargement to correspond with the almost functionless wings. The 'lumbar brain,' on the other hand, extends through eleven segments and its transverse section has an average area of 38.5 sq. mm., which is 20.3 sq. mm. greater than the largest area from the cervical enlargement. Hardesty ('05) finds a similar condition in the cord of the emu, a bird closely allied to the ostrich. The ostrich and emu may be taken as examples in the bird family, of a condition present in the kangaroo among mammalia.

Spitzka ('86) states that the bat has an insignificant lumbar enlargement to correspond with the diminutive posterior extremities. The specimen of bat here used (*Nyctinomus brasiliensis nasutus*) has a well marked enlargement in the lumbar region as well as a large cervical enlargement which furnishes innervation for its powerful wings (figs 24, *C* and *L*). Likewise, it is interesting to note, as stated by Cunningham ('09), that in the cetacea, although there are no visible hind limbs there is a well marked lumbar enlargement. In these animals, this enlargement is no doubt required by the large and powerful tail.

The grey substance

The *H* shape of the grey figure, described as characteristic of the human cord, holds, as is well known, for the great majority of mammals. In the thoracic region of the bear, rhesus monkey, cat, dog, lynx, and spider monkey the *H* is highly modified (*Th* in figs. 4, 10, 12, 14, 16, 17). In the first five animals the peculiar shape consists in a shortening or flattening of the dorsal horns and a relatively very wide grey commissure. The almost complete absence of the dorsal horns in these animals

TABLE 6

Arcus of transverse sections in square millimeters. Table 6 records the total areas in square millimeters of the transverse sections from the different regions of the spinal cords of the animals given and actual areas of the portions of the transverse sections as indicated in the headings of the columns.

ANIMAL	REGION	1 TOTAL AREA	2 AREA OF GREY FIGURE	3 AREA OF DORSAL FUNICULUS	4 AREA OF ANTERO- LATERAL FUNICULUS	5 TOTAL AREA OF WHITE SUBSTANCE
Elephant.....	C.	429.68	74.06	100.00	255.62	355.62
	T.	224.06	16.40	40.93	166.73	207.66
	L.					
Horse.....	C.	262.96	44.53	47.34	171.09	218.43
	T.	131.25	14.06	15.15	102.04	117.19
	L.	233.35	62.50	51.56	119.29	170.85
Ox.....	C.	167.81	36.87	29.06	101.88	130.94
	T.	85.93	9.37	9.68	66.88	76.56
	L.	132.03	39.53	21.87	70.63	92.50
Bear.....	C.	120.15	29.92	20.70	69.53	90.23
	T.	44.03	4.92	7.57	31.54	39.11
	L.	74.60	21.56	16.64	36.40	53.04
Hog.....	C.	85.00	18.59	11.59	54.82	66.41
	T.	36.40	4.14	4.92	27.34	32.26
	L.	59.92	11.25	13.43	35.24	48.67
Man.....	C.	100.39	16.75	26.17	57.47	83.64
	T.	52.42	5.00	14.53	32.89	47.42
	L.	65.93	21.12	15.62	29.18	44.81
Sheep.....	C.	82.65	21.71	11.56	49.38	60.94
	T.	35.70	4.37	3.12	28.21	31.33
	L.	61.70	20.15	9.21	32.35	41.56
Kangaroo.....	C.	55.93	10.62	9.60	35.71	45.31
	T.	40.46	3.75	4.21	32.50	36.71
	L.	69.92	19.68	12.42	37.82	50.24
Ape.....	C.	41.64	10.23	8.82	22.59	31.41
	T.	16.25	1.56	3.12	11.57	14.69
	L.	26.09	10.54	4.60	10.95	15.55
Dog.....	C.	35.46	10.93	5.78	18.75	24.53
	T.	14.21	2.65	1.56	10.00	11.56
	L.	26.25	9.68	3.90	12.67	16.57
Fox.....	C.	45.15	11.79	6.25	27.11	33.36
	T.	21.40	2.26	2.03	17.11	19.14
	L.	37.18	11.71	4.68	20.79	25.47
Lynx.....	C.	80.37	22.03	14.06	44.30	58.36
	T.	27.89	3.20	3.20	21.49	24.69
	L.	53.90	21.25	9.29	23.36	32.65

TABLE 6—Continued

ANIMAL	REGION	1 TOTAL AREA	2 AREA OF GREY FIGURE	3 AREA OF DORSAL FUNICULUS	4 AREA OF ANTERO- LATERAL FUNICULUS	5 TOTAL AREA OF WHITE SUBSTANCE
Raccoon.....	C.	45.39	11.87	10.70	22.82	33.52
	T.	17.73	1.79	3.82	12.12	15.94
	L.	33.82	12.65	5.39	15.78	21.17
Rhesus monkey.....	C.	26.95	9.06	5.62	12.17	17.89
	T.	12.81	1.56	1.54	9.71	11.25
	L.	21.87	8.43	4.37	9.07	13.44
Opossum.....	C.	23.90	6.40	4.68	12.82	17.50
	T.	10.78	1.56	1.56	7.66	9.22
	L.	20.85	8.04	2.50	10.35	12.81
Cat.....	C.	35.31	10.56	5.70	19.05	24.75
	T.	15.15	2.10	2.93	10.12	13.05
	L.	28.20	10.46	4.92	12.82	17.74
Spider monkey.....	C.	32.81	12.03	6.25	14.53	20.78
	T.	13.90	2.18	3.12	8.60	11.72
	L.	25.00	10.85	4.53	9.62	14.15
Java monkey.....	C.	30.46	9.37	5.46	15.63	21.09
	T.	11.09	1.40	1.25	8.44	9.69
	L.	23.28	11.71	2.34	9.23	11.57
Rabbit.....	C.	21.32	6.40	2.81	12.11	14.92
	T.	13.59	2.50	1.56	9.53	11.09
	L.	20.62	7.81	2.57	10.24	12.81
Agouti.....	C.	35.00	8.20	6.71	20.09	26.80
	T.	21.87	2.65	3.59	15.63	19.22
	L.	34.21	15.31	4.92	13.98	18.90
Guinea-pig.....	C.	9.92	3.12	1.48	5.32	6.80
	T.	6.71	1.71	1.09	3.91	5.00
	L.	9.84	4.45	1.40	3.99	5.39
Gray rat.....	C.	11.32	4.06	1.79	5.47	7.26
	T.	6.09	1.87	1.01	3.21	4.22
	L.	9.37	3.82	1.40	4.15	5.55
Mouse.....	C.	3.98	1.87	0.63	1.48	2.11
	T.	2.03	0.78	0.31	0.94	1.25
	L.	3.43	1.56	0.70	1.17	1.87
Bat.....	C.	3.59	2.18	0.41	1.00	1.41
	T.	1.71	1.32	0.12	0.27	0.39
	L.	2.96	1.56	0.48	0.92	1.40

is quite striking. This condition may be interpreted as a dorso-ventral thickening and mesial fusion of the dorsal horns (posterior grey columns) since the gelatinous substance of Rolando and the dorsal horn cells are present as normally. The spider monkey (fig. 17) possesses relatively short ventral or anterior horns and this relative shortening is evident through all three regions of the cord.

In all the animals here studied, with one exception, the width or thickness of the ventral horn in the cervical region exceeds that of the dorsal horn. This exception is in the kangaroo in the cervical region of which, the caput of the dorsal horn is wider than is the ventral horn. This condition in the kangaroo is due, not to a relatively extra wide dorsal horn but to a narrow ventral horn. Because of its small anterior extremities, it is probable that cutaneous innervation may not have decreased to the same extent as the innervation required by the muscles, which receive motor or ventral root-fibers and which have atrophied through greatly lessened use. If such be the case, the retained width of the dorsal horn may be understood in that it contains the cell bodies of association and commissural neurones about which the dorsal root or sensory fibers terminate for purposes of functionally associating different levels and the two sides of the spinal cord with sensations brought into the cervical region.

The lumbar region shows throughout the series the ventral horn to be wider than the dorsal horn (columns 7 and 8, table 4). However, the average difference in the width of the two horns in the lumbar region for the series is only 0.52 mm. while in the cervical region the average difference is 0.64 mm., the ventral horn being the wider.

The difference in width of the dorsal and ventral horns is in the thoracic region very much less than in either of the other two regions (columns 7 and 8, table 3). In most cases the dorsal horn is somewhat the wider. This is what we should expect since in the thoracic region the musculature is lessened in amount to an extent greater than is the sensory area decreased.

The dorso-ventral width of the grey commissure as taken through the central canal (column 9, tables 2, 3, 4), varies through-

out the series. The average thickness is greater in the thoracic region than in either of the other two regions, being 0.84 mm. in the thoracic, 0.61 mm. for the cervical and 0.54 mm. for the lumbar region. It is noticeably thick in the thoracic regions of the ox, bear, hog, sheep, kangaroo, fox, Java monkey, and rabbit.

Reticular formation. There is considerable variation in the amount of reticular formation as shown in the figures. This reticular network is believed to be formed, at least in part, by a dispersion of the lateral portions of the grey figure (1) by bundles of longitudinally coursing association fibers (*fasciculi proprii*), (2) by fibers passing out of the grey figure into the white substance and (3) by the fibers from the crossed pyramidal tracts leaving the lateral funiculus and entering the grey figure to terminate about ventral horn cells. However, the lateral pyramidal tract may have little to do with it for the animals which have their pyramidal tract in the dorsal funiculus have the reticular formation as well, often better, marked than do animals which have the pyramidal tract in the lateral funiculus.

Nucleus dorsalis. A nucleus dorsalis (Clarke's column) is well marked in the thoracic region of by far the greater number of animals here studied. In the following six mammals, kangaroo, opossum, agouti, guinea-pig, rat, and mouse the nucleus is not clearly defined. As will be mentioned, the pyramidal tract in each of these animals courses, probably in the dorsal funiculus and it may be that the dorsal position of this tract is in some way associated with the modified appearance of the nucleus dorsalis. These observations are based on the study of Weigert sections in which the cell-bodies are not stained, but the position and usually a very good outline of the cell-body may be seen.

Proportion of grey substance to white. An idea of the relative amount of grey substance as compared with white in the spinal cord is best obtained from a study of the ratios of the absolute areas of the two. Table 7 gives such a ratio for the three regions of the cord of each animal. The area of the grey figure is to the total area of the white substance as 1 is to the figure given in the table. All of the spinal cords, with the exception of

the mouse and bat, show the lumbar region to contain the largest relative amount of grey substance, while the thoracic contains the smallest relative amount, in each animal. An average of the ratios in the three regions is valuable since it provides a comparison of the relative amounts of white and grey substance through, what we may consider, the entire cord. The average of the ratios in the three regions emphasizes the well known fact that, in general, the smaller the animal, the greater is the proportion of grey substance to white. A glance at the drawings of the cervical and thoracic regions of the elephant (fig. 1, *C* and *Th*) and the corresponding regions of the bat (fig. 24, *C* and *Th*) show the relatively high proportion of grey substance in the smaller animals.

TABLE 7

Ratio of area of grey substance to area of white substance. Table 7 records the ratios of grey substance to white substance obtained by dividing the figures in column 2 of table 6 into those of column 5, table 6, that is to say, the area of grey substance is to the area of white substance as 1 is to the figures recorded in columns 1, 2, and 3.

ANIMAL	CERVICAL 1	THORACIC 2	LUMBAR 3	AVERAGE 4
Elephant.....	4.8	12.6		
Horse.....	4.9	8.3	2.7	5.2
Ox.....	3.6	6.1	2.3	4.0
Bear.....	3.0	7.9	2.4	4.4
Hog.....	3.6	7.8	4.3	5.2
Man.....	5.0	9.5	2.1	5.5
Sheep.....	2.8	7.2	2.1	4.0
Kangaroo.....	4.3	9.8	2.6	5.5
Ape.....	3.1	9.4	1.5	4.6
Dog.....	2.2	4.3	1.7	2.7
Fox.....	2.8	8.5	2.2	4.5
Lynx.....	2.6	7.7	1.5	3.9
Raccoon.....	2.8	8.9	1.7	4.4
Rhesus monkey.....	2.0	7.2	1.6	3.6
Opossum.....	2.7	5.9	1.6	3.4
Cat.....	2.3	6.2	1.7	3.4
Spider monkey.....	1.7	5.4	1.3	2.8
Java monkey.....	2.3	6.9	0.9	3.3
Rabbit.....	2.3	4.4	1.6	2.7
Agouti.....	3.3	7.3	1.2	3.9
Guinea-pig.....	2.2	2.9	1.2	2.1
Rat.....	1.8	2.3	1.5	1.8
Mouse.....	1.1	1.6	1.2	1.3
Bat.....	0.6	0.3	0.9	0.6

White substance

Dorso-intermediate sulcus. In the kangaroo, raccoon, opossum, and Java monkey, a dorso-intermediate sulcus is clearly present in all three regions (figs. 8, 13, 15 and 18). In a much larger number of species it is clearly evident only in the cervical region, man being among this number. In some animals, the ox, sheep and dog, for example, in Weigert preparations, it is not to be seen in any of the regions. In most of the sections, considerable difference is noted between the areas of white substance on either side of this sulcus. The area nearest the dorsal median septum, corresponding to the fasciculus gracilis in man, is composed of relatively small, closely packed axones which give it a darkened appearance in transverse sections stained by the Weigert method. The lateral area which corresponds to man's fasciculus cuneatus, is composed of relatively larger axones. Singer ('81), who describes the origin and position of the fasciculus gracilis in the dog, shows in his drawings no septum between it and the fasciculus cuneatus. It must be that in certain animals the factors which determine whether there shall be an ingrowth of the pial connective tissue to form this sulcus are different during fetal life while the fasciculus gracilis and fasciculus cuneatus are being acquired and becoming medullated. In most cases where the sulcus is wanting, one may observe in the cervical and thoracic regions that the fibers are smaller and more closely accumulated, and that the area is darker, near the dorsal median septum than in the more lateral areas of the dorsal funiculus.

Position of pyramidal tract. Simpson ('02) describes the pyramidal tract for the dog, cat and monkey as situated in the dorsal part of the lateral funiculus. The guinea-pig and mouse, according to Reverly and Simpson ('10), who confirm the earlier work of Von Bechterew and Von Lenhossek, have the pyramidal tract in the dorsal funiculus. King ('10) has traced the pyramidal tract of the rat into the dorsal funiculus. Spitzka ('86) states that the sheep and ox have no fibers, to be seen macroscopically, which cross from the pyramids in the medulla oblongata into the lateral funiculus. The elephant, according

to Hardesty ('02), has a part at least of the pyramidal tract situated on either side of the mid-line between the dorsal and ventral lamina of the grey commissure. Believing that it corresponds to the lateral or crossed pyramidal tract in man, he has designated it 'fasciculus cerebro-spinalis internus' (fig. 1, *C* and *Th*). Burkholder ('04) describes this tract in the sheep as occupying the same position as in the elephant and has termed it the 'fasciculus cerebro-spinalis internus' after Hardesty. King and Simpson ('10) state that the pyramidal fibers for the sheep are situated in the reticular formation in the lateral aspect of the dorsal horn. The specimen of sheep here studied, as well as the ox, presents in all three regions a structure identical in position to that described by Burkholder (figs. 3 and 7, *C*, *Th* and *L*). Symington ('08) states that the kangaroo has the pyramidal tract in the dorsal funiculus and the rabbit has the tract in the lateral funiculus.

I have compared sections through the pyramidal decussation in the medulla of the agouti and opossum with those of the rat, mouse and kangaroo, and am of the opinion that the pyramidal tract in the former animals, as well as in the latter, is situated in the dorsal funiculus. The agouti, like the rat, mouse and guinea-pig, is a rodent, while the opossum, being a marsupial, is related to the kangaroo. From an examination of sections through the medulla of the raccoon and of the fox, the pyramidal fibers appear to course in the reticular formation. However, the experimental method is the only trustworthy one for determining the position of any fiber tract.

Comparison of funiculi. As is to be expected, all of the cords here considered have, in each region, a dorsal funiculus which is exceeded in actual area in transverse section by the ventro-lateral funiculus (table 6). The comparative size of the funiculi can be best expressed in the form of a ratio. Table 8 (*a* and *b*) record such ratios for each region, obtained by dividing the area of the ventro-lateral funiculus by that of the dorsal funiculus. It is clear that the higher the ratio, the smaller relatively is the dorsal funiculus. For example, table 8a, column 4, gives man as having the lowest average ratio, 2.10, which

means that the average size of the ventro-lateral funiculus for the three regions is 2.10 times the size of the dorsal funiculus for the three regions. The fox with the highest average ratio, 5.73, shows therefore relatively the smallest dorsal funiculus of the series.

While the human cord does not show the largest relative size of the dorsal funiculus in either the cervical or the lumbar region, being surpassed in the former by the raccoon and rhesus monkey, in the latter by mouse and rhesus monkey, yet the average of the three regions places man first. In other words, all the other

TABLE 8 a

Ratios of dorsal funiculus to ventro-lateral funiculus. Table 8a has been computed from data in table 6, by dividing the figures in column 4 by those in column 3, table 6. In other words, the dorsal funiculus is to the ventro-lateral funiculus as 1 is to the figures in columns 1, 2 and 3 below. Column 4 records the average ratios of 1, 2 and 3. The animals are arranged according to body weight.

ANIMAL	CERVICAL 1	THORACIC 2	LUMBAR 3	AVERAGE RATIO 4
Elephant.....	2.55	4.07		
Horse.....	3.61	6.73	2.31	2.96
Ox.....	3.50	6.90	3.22	4.54
Bear.....	3.35	4.16	2.18	3.23
Hog.....	4.72	5.55	2.62	4.29
Man.....	2.19	2.26	1.86	2.10
Sheep.....	4.27	9.04	3.51	5.60
Kangaroo.....	3.71	7.72	3.04	4.82
Ape.....	2.56	3.70	2.38	2.88
Dog.....	3.24	6.41	3.24	4.29
Fox.....	4.33	8.42	4.44	5.73
Lynx.....	3.15	6.71	2.51	4.12
Raccoon.....	2.13	3.17	2.92	2.74
Rhesus monkey.....	2.18	7.03	1.75	3.65
Opossum.....	2.52	4.91	4.12	3.85
Cat.....	3.34	3.45	2.60	3.13
Spider monkey.....	2.32	2.75	2.12	2.39
Java monkey.....	2.88	6.75	3.94	4.52
Rabbit.....	4.30	6.10	3.98	4.79
Agouti.....	2.99	4.35	2.84	3.39
Guinea-pig.....	3.59	3.58	3.58	3.58
Rat.....	3.05	3.17	2.90	3.04
Mouse.....	2.34	3.03	1.67	2.34
Bat.....	2.43	2.27	1.91	2.20

cords in the series have average dorsal funiculi smaller in proportion to the ventro-lateral funiculi than does the human spinal cord. The dorsal funiculus in man is made up, in large part, of ascending axones of spinal ganglion neurones, a large proportion of which connect the cord with the encephalon. The latter reaches its highest development in man and the dorsal funiculus is correspondingly increased in size. We might expect the cervical region in man especially to show a larger ratio for the dorsal funiculus than does the thoracic or lumbar region since

TABLE 8 b

Ratios dorsal funiculus to ventro-lateral funiculus. Table 8b records the same data as table 8a but with the animals arranged according to the relative size of the dorsal funiculus in each region of the cord instead of according to body weight.

CERVICAL 1	THORACIC 2	LUMBAR 3	AVERAGE 4
Raccoon.....2.13	Man.....2.26	Mouse.....1.67	Man.....2.10
Rhesus monkey..2.18	Bat.....2.27	Rhesus mon-	Bat.....2.20
Man.....2.19	Spider mon-	key.....1.76	Mouse.....2.34
Spider monkey...2.32	key.....2.75	Man.....1.86	Spider mon-
Mouse.....2.34	Mouse.....3.03	Bat.....1.91	key.....2.39
Bat.....2.43	Raccoon...3.17	Spider mon-	Raccoon....2.75
Opossum.....2.52	Rat.....3.17	key.....2.12	Ape.....2.88
Elephant.....2.55	Cat.....3.45	Bear.....2.18	Horse.....2.95
Ape.....2.56	Guinea-pig.3.58	Horse.....2.31	Rat.....3.04
Java monkey....2.88	Ape.....3.70	Ape.....2.38	Cat.....3.13
Agouti.....2.99	Elephant..4.07	Lynx.....2.51	Bear.....3.23
Rat.....3.05	Bear.....4.16	Cat.....2.60	Agouti.....3.39
Lynx.....3.15	Agouti....4.35	Hog.....2.62	Guinea-pig.3.58
Dog.....3.24	Opossum..4.91	Agouti....2.84	Rhesus mon-
Cat.....3.34	Hog.....5.55	Rat.....2.90	key.....3.65
Bear.....3.35	Rabbit...6.10	Raccoon...2.92	Opossum...3.85
Ox.....3.50	Dog.....6.41	Kangaroo..3.04	Lynx.....4.12
Guinea-pig....3.59	Lynx.....6.71	Ox.....3.22	Dog.....4.29
Horse.....3.61	Horse.....6.73	Dog.....3.24	Hog.....4.29
Kangaroo.....3.71	Java mon-	Sheep.....3.51	Java mon-
Sheep.....4.27	key.....6.75	Guinea-pig.3.58	key.....4.52
Rabbit.....4.30	Ox.....6.90	Java mon-	Ox.....4.54
Fox.....4.33	Rhesus mon-	key.....3.94	Rabbit....4.79
Hog.....4.72	key.....7.03	Rabbit....3.98	Kangaroo...4.82
	Kangaroo..7.72	Opossum..4.12	Sheep.....5.60
	Fox.....8.42	Fox.....4.44	Fox.....5.73
	Sheep.....9.04		

the two fasciculi of which it is largely composed contain a large proportion of spino-cerebral fibers ascending from all segments of the cord and thus it must increase in size from below upward. However, column 3, table 8a, shows the lumbar region of the human cord to possess a larger relative size for the dorsal funiculus than either of the other two regions. The pyramidal tract, in the ventro-lateral funiculus, which has its maximum size in the cervical region is one of the principal factors in reducing the relative size of the dorsal funiculus in this region. Another factor in the lumbar enlargement is the large proportion of association fibers in the dorsal fasciculi proprii of the dorsal funiculus.

In those animals in which the crossed pyramidal tracts course in the dorsal, instead of in the lateral funiculus, one would expect a relatively larger area of dorsal funiculus and a resultingly smaller area in the ventro-lateral funiculus. Reference to these animals in tables 8a and 8b does not show a relatively greater area of the dorsal funiculus (smaller ratio of the ventro-lateral funiculus) to be of distinguishingly constant occurrence. In some cases, agouti, guinea-pig and rat, for example, the ratios for the cervical region are approximately the same as those for the lumbar region in spite of the fact that there are both ascending and descending fibers in the dorsal funiculus which must increase as the cord is ascended.

In all mammals, there is a decrease in the absolute area of the dorsal funiculus in passing from the lumbar to the thoracic region. The horse, ox, bear, sheep, kangaroo, hog and lynx show a very great decrease in the area of the dorsal funiculus from the lumbar to the thoracic region (table 6). The difference in the area of the ventro-lateral funiculus in the two regions of the above animals is very slight. This fact is expressed in another form in columns 2 and 3, table 8b. The horse, for example, has in the transverse section of the lumbar region a ventro-lateral funiculus 2.31 times that of the dorsal funiculus, while in the thoracic region, the ventro-lateral funiculus is 6.73 times the dorsal. It is probable that in these animals an especially large number of axones from the dorsal roots of the lumbar region extend up the cord only a short distance and then terminate around cell-bodies in the grey substance.

The ape and monkeys, as expected from their high position among mammals, show a relatively large dorsal funiculus. The spider monkey, for example, has an average ratio for the three regions of 2.39, that is to say, the dorsal funiculus of the spider monkey is only slightly less than one-half the size of the ventro-lateral. The relatively large dorsal funiculus of this animal is explained in part at least by the fact that it possesses an exceptionally long, large and specially functioning tail whose skin area must require a large additional number of ascending axones in the cord. The striking motility and functional control of this tail must mean additional and no doubt a special bundle of pyramidal axones. These latter, descending in the lateral funiculus, render the dorsal funiculus only relatively less large than it would be otherwise.

I am not able to explain why the cord of the raccoon should show such a relatively large dorsal funiculus which, in the cervical region is to the ventro-lateral funiculus as 1 is to 2.13, being relatively larger in this region than that found in any of the other animals. The upper extremities of the raccoon are somewhat less highly functional than those of the monkey, its tail is relatively no larger and but little more functional than that of the dog or fox and its pyramidal tract does not appear to course in its dorsal funiculus. The mouse and agouti have relatively large dorsal funiculi, due, at least in part, to the fact that they have the crossed pyramidal tract in this funiculus.

Column 4, table 8b, gives the fox as having an average dorsal funiculus less than one-fifth the size of the ventro-lateral. This must mean that he possesses an enormous number of association fibers in the lateral funiculus or that the spino-cerebellar fasciculi are unusually large. It is to be noted that the great variation in relative size of the two funiculi which most of the animals show throughout the three regions is not evident in the fox (table 8b). The acuteness of the fox in reflex action is proverbial and complex reflex activities are anatomically explained on the basis of abundant association fibers (fasciculi proprii). Also, for cerebral muscular control, this animal may have relatively large pyramidal fasciculi. The sheep and rabbit likewise are popu-

larly known as reflex animals. They stand close to the fox in the small size of the dorsal funiculus as compared with the ventrolateral. It is probable that, in these animals, by far the greater number of dorsal root axones terminate in the grey substance of the cord after a comparatively short course in the dorsal funiculus, thus accounting, in large part, for the small size of the latter. Man and the monkeys, as examples of animals with a highly developed encephalon, have relatively large dorsal funiculi; while the fox, sheep, and rabbit with acute spinal reflexes have very small dorsal funiculi. The relative size of the dorsal and ventrolateral funiculi, however, may be the result of many factors of which we have considered but a few.

This investigation has been carried on under the direction of Prof. Irving Hardesty who kindly furnished much of the material. I am under obligations to him for many helpful suggestions.

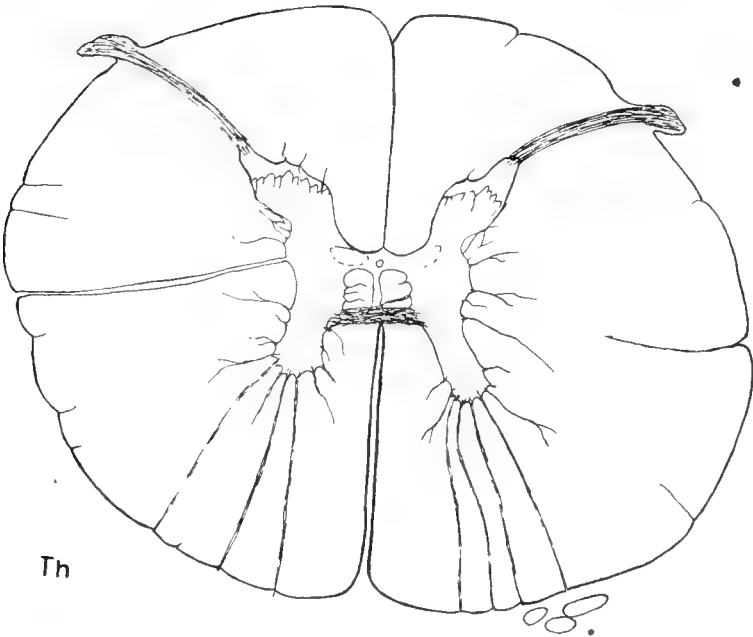
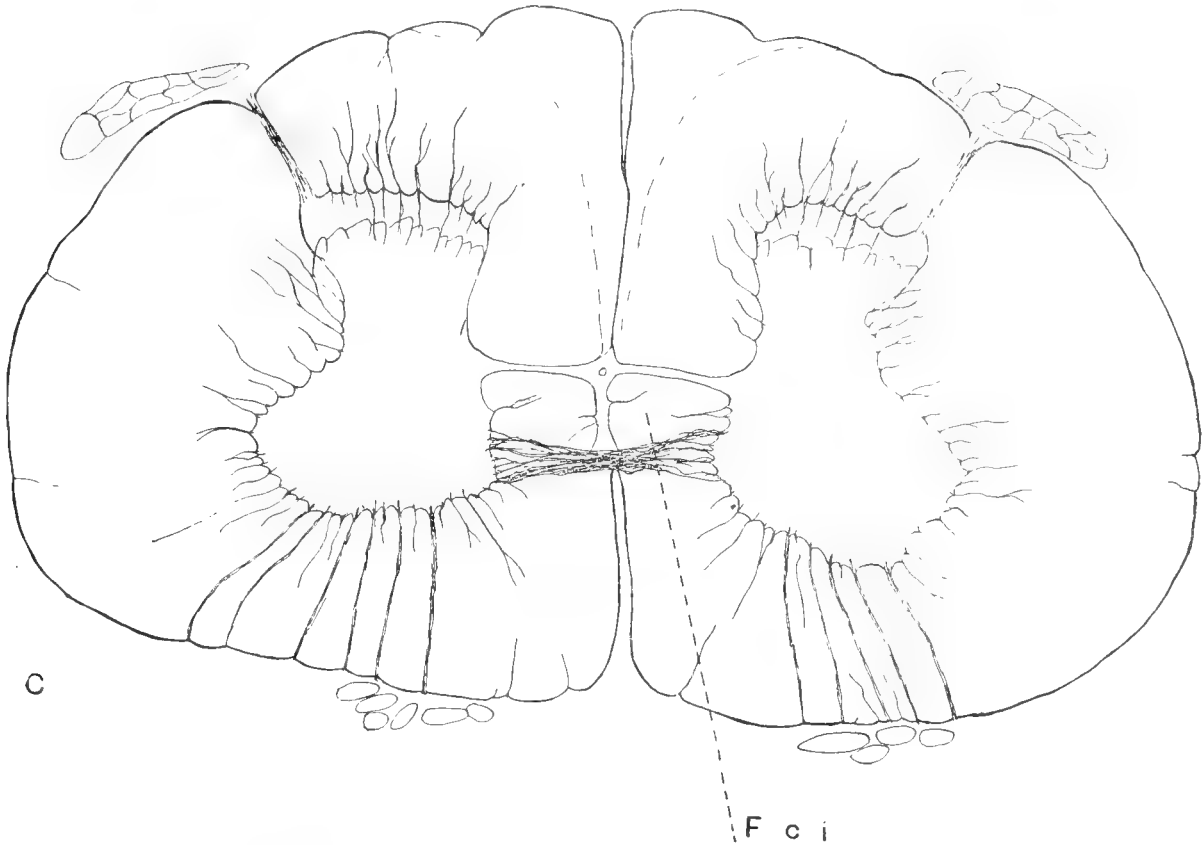
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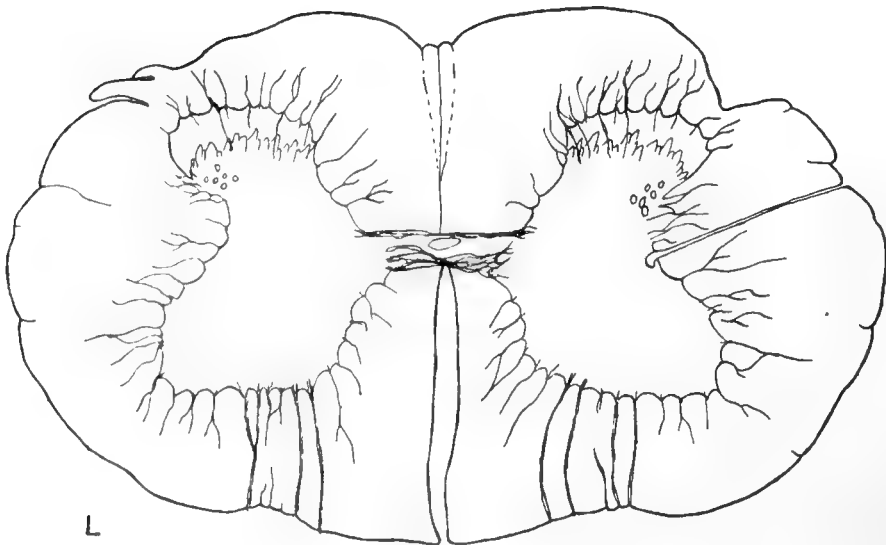
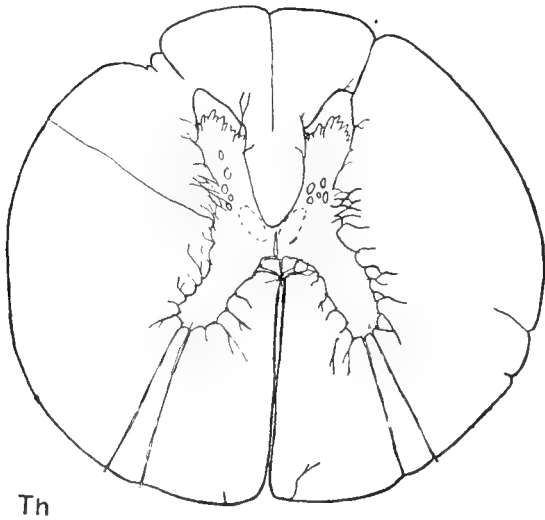
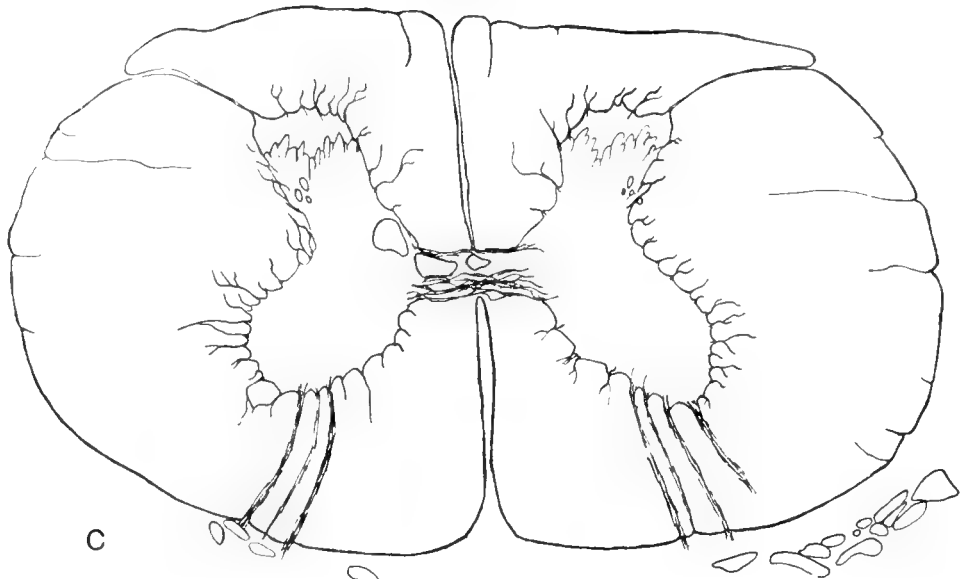
EXPLANATION OF PLATES

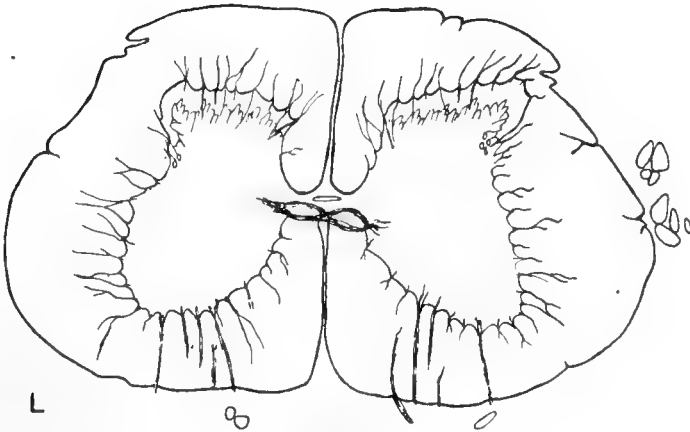
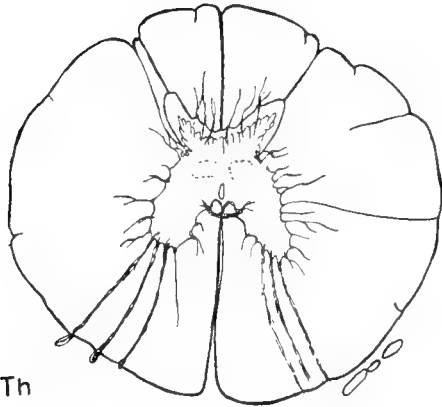
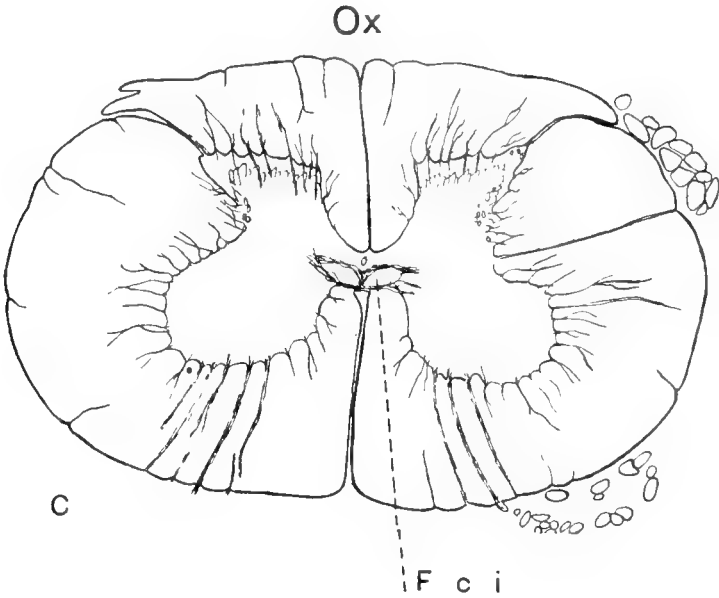
The plates contain figures representing outline drawings of transverse sections taken from the sixth cervical, eighth thoracic, and third lumbar segments of the spinal cords of the twenty-four mammals named. The drawings were traced from projections of the respective section with the Edinger Drawing Apparatus set for a magnification of eight diameters. They are here reduced one-half and thus are magnified four times. The letter *C* indicates the cervical region, *Th*, the thoracic, and *L*, the lumbar; *F.c.i.*, fasciculus cerebro-spinalis internus.

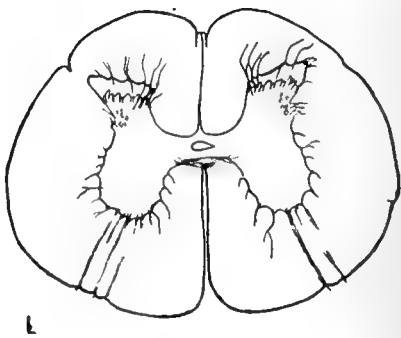
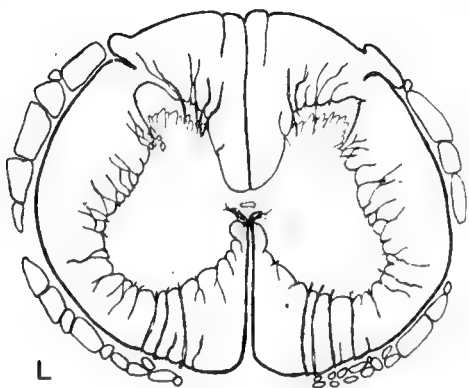
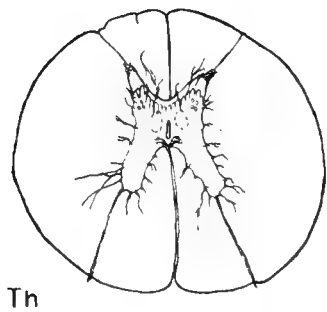
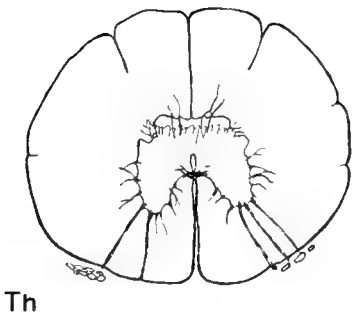
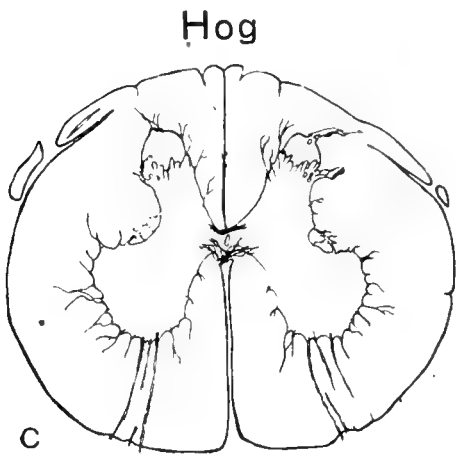
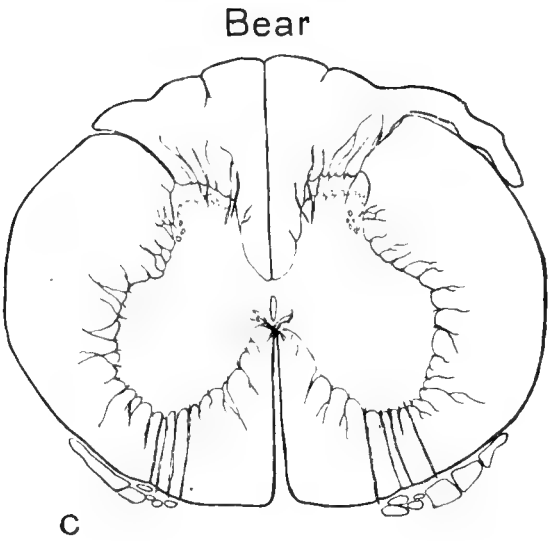
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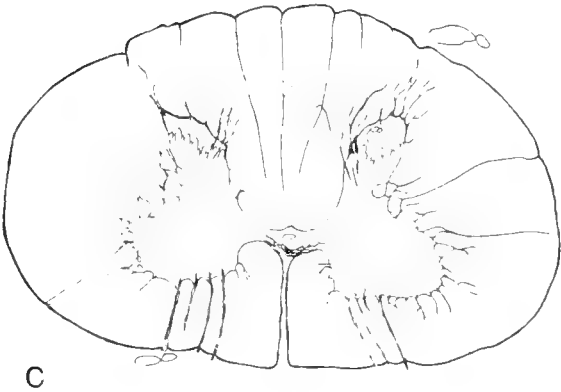
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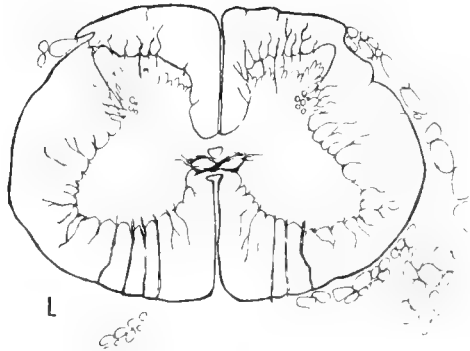
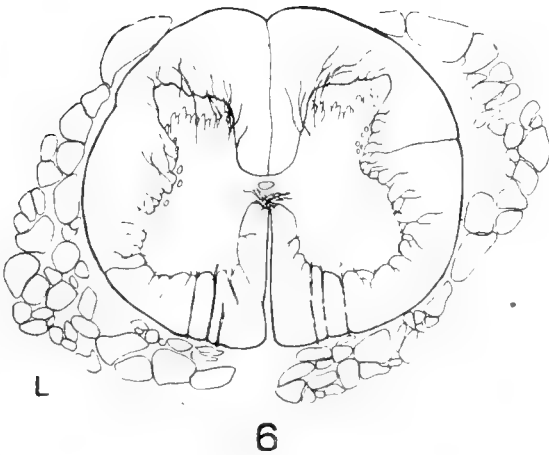
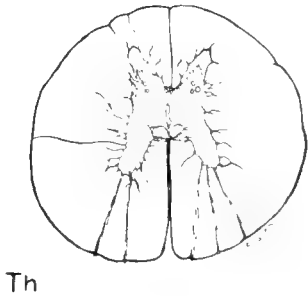
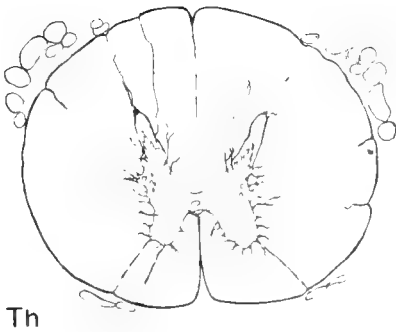
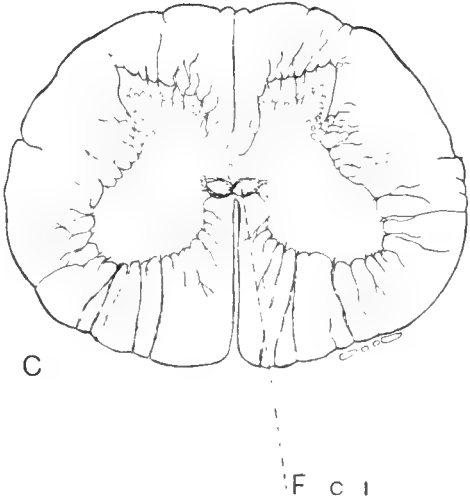




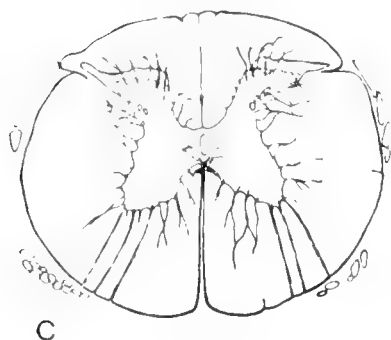
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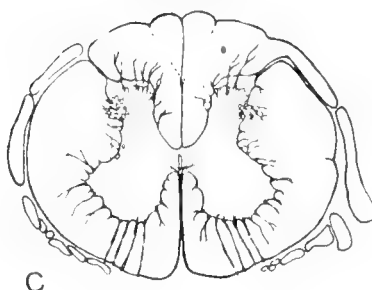
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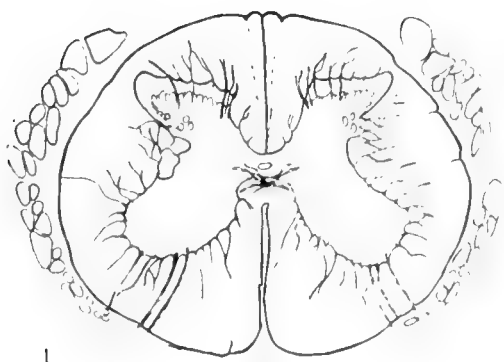
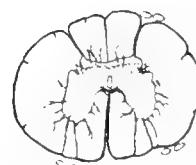
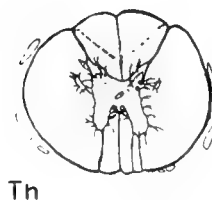
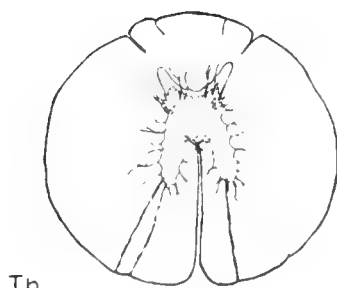
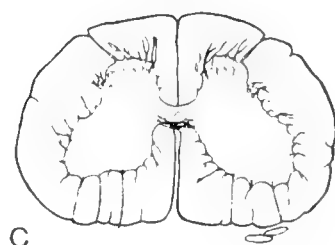
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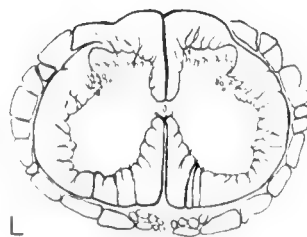
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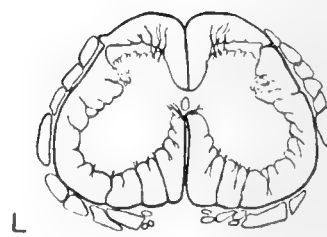
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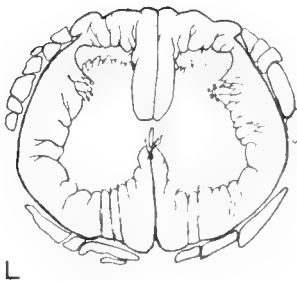
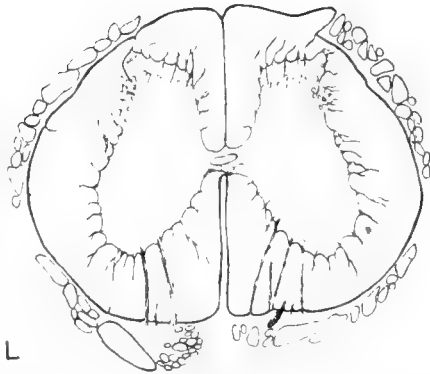
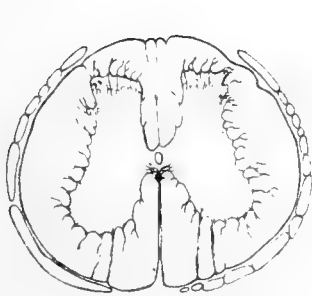
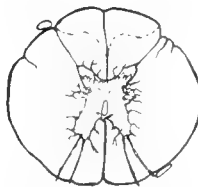
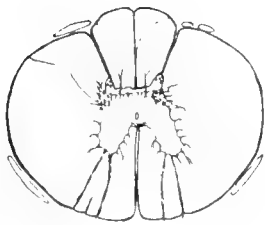
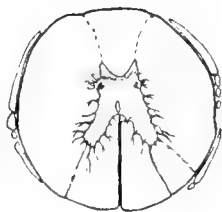
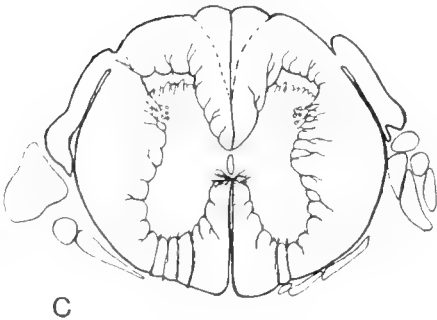
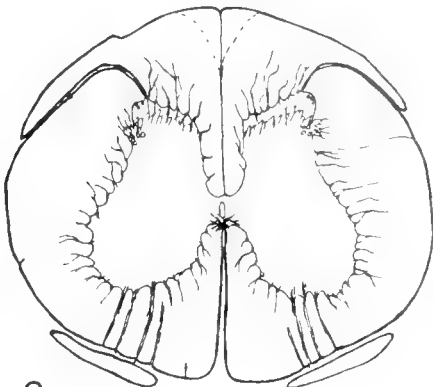
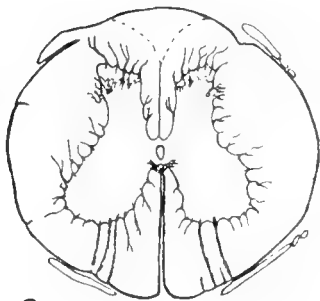


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Lynx

Raccoon

Fox

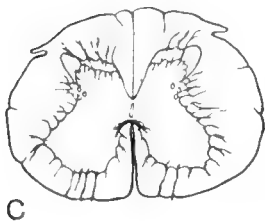


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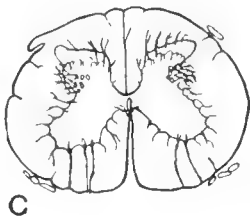
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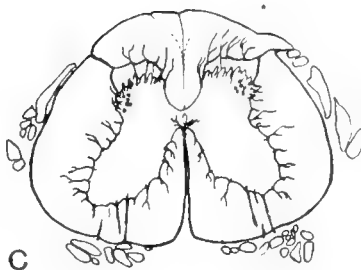
Rhesus
monkey



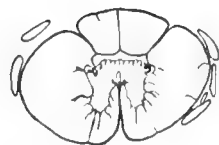
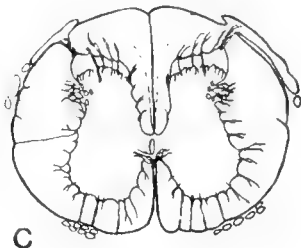
Opossum



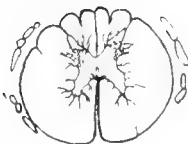
Cat



Spider
monkey



Th



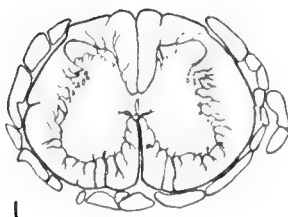
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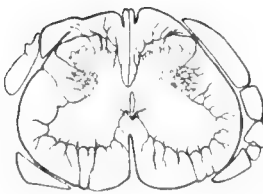
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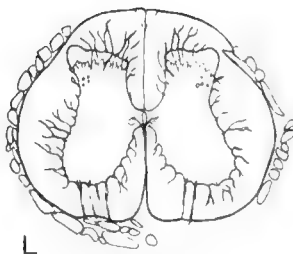
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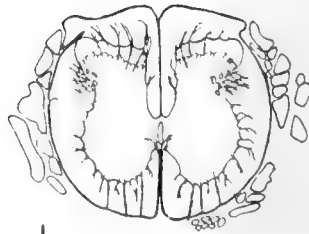
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THE DEVELOPMENT OF THE CEREBRAL CORTEX

E. LINDON MELLUS

From the Anatomical Laboratory of the Johns Hopkins University

TWO FIGURES

During the course of an investigation (still incomplete) of the so-called 'motor area' in man (anterior central convolution of the cerebral cortex) I had occasion to examine sections from that area in the brain of an eight months foetus. To my surprise I found the corona radiata of both central convolutions thickly sown with what appeared to be migrating cells. These cells were in various stages of development, the large majority resembling the neuroblasts just leaving the matrix in earlier stages, but many were well advanced in development, the nucleus having a distinct nucleolus and being enveloped by a considerable cell body. This cell body was either round or ovoid. Many of the latter form had a distinct apical process at one or the other extremity, this being sometimes directed toward the cortex, at other times away from it as if the nucleus were being propelled so rapidly that the enveloping protoplasm was inclined to drag behind. The long diameter of the ovoid cells seemed always to indicate the direction of the movement, some of those quite near the cortex of the fissure walls having apparently turned toward the cortex and in such the long diameter was at an acute or almost right angle to that of those more toward the center of the corona radiata. The granules or undeveloped nuclei scattered through the corona radiata were of two sorts and sizes. The smaller about 5 micra in diameter stained deeply and showed no nucleolus, the others about 10 micra in diameter were faintly stained and contained two or more dark spots. At first I was inclined to look upon the smaller of these two granules as spongioblasts and the paler as neuroblasts, but I found as many of the latter as the former in the first (molecular) layer of the cortex. In this por-

tion of the cortex I find the line of demarkation between the first or molecular and the second (external granular layer of Meynert) very striking, composed almost entirely of small round deeply stained granules. This line is less pronounced on the crest of the convolution than within the fissure, both on the walls of the fissures and at the base. The stain is most intense at the base of the fissure and becomes gradually paler as we approach the surface of the brain. The sharp definition of this line seems to be due partly to the deeper stain taken by these small granules and partly to their being very closely packed. But on the crest of the convolution the stain of the same cells is distinctly fainter.

The appearance of so many cells in the white matter at this late stage of intra-uterine life naturally led to further investigation. The available material was by no means perfect. It consisted of a somewhat fragmentary brain from which the brain stem and the basal ganglia had been removed, and the least injured portion of the ventricle was the occipital end. The matrix surrounding the posterior horn of the lateral ventricle was in the greatest activity, throwing off neuroblasts in enormous numbers (fig. 1). There was still present between the ventricle and the cortex the layer of neuroblasts called by His the 'Übergangschicht.' Between this and the cortex the corona radiata was full of partly developed cells and naked nuclei apparently streaming towards the cortex in more or less radial lines. From the matrix broad streams of nuclei led more or less directly to the 'Übergangschicht.' These streams were not everywhere radially directed, but for a certain distance ran parallel to the wall of the ventricle. In cross section the formation of the 'Übergangschicht' was distinctly outlined, completely surrounding the ventricle except in that portion contiguous to the calcarine fissure, where the matrix more nearly approached the resting stage. Here the cortex of the calcarine fissure in its entire extent was much more deeply stained than the cortex on the external surface of the convolutions and the 'Übergangschicht' was not present. At each extremity of the long narrow slit representing the posterior horn of the lateral ventricle the broad band of deeply stained nuclei is a prominent object in the section clearly visible to the naked eye.

From the outer edges of this band radially directed streams of nuclei could be followed into the hiatus of each contiguous convolution. Sagittal sections through the occipital operculum showed similar conditions, the ventricle surrounded by this broad band of deeply stained nuclei from the external borders of which streams of cells radiated toward the surrounding cortex.



Fig. 1 Transverse section of occipital lobe of the brain of an eight-months human embryo. Enlarged 2 \times . C, calcarine fissure; V, posterior horn of lateral ventricle.

A frontal section through the temporal region about midway between the frontal and occipital pole showed the matrix surrounding the ventricle to be in a state of great activity. The 'Übergangschicht' is here distinctly stratified and the four layers described by His as developing between the third and fourth month can be easily seen. The white matter between the closely packed collection of neuroblasts and the cortex is thickly strewn with migrating cells.

Owing to imperfections in the material, the relations in the frontal lobes were not so clear but the number of migrating cells passing in the white matter was quite as great as in other parts of the brain. In addition to this there were numerous masses of nuclei here and there distinctly visible to the naked eye. The nuclei were thickly massed and resembled both the 'Übergangschicht' and the streams seen passing from the ventricle in other parts of the brain.

A portion of the right hemisphere of a new-born child (still-born) shows the same activity in the production of neuroblasts by the germinal cells in the walls of the ventricle. The stratification of the calcarine cortex is more marked than in the eight months brain but the 'Übergangschicht' surrounding the ventricle in the occipital lobe is still distinct and at some points it consists of two layers separated by a pale layer. From the 'Übergangschicht' several quite distinct streams of neuroblasts are directed toward the various convolutions and can be followed as such for some distance. The cells of the calcarine cortex were distinctly more developed in the new-born than in the eight months brain. While in the latter the solitary cells of Meynert were the only ones with a distinct cell body, in the former a majority of the cells of the calcarine cortex had developed distinct processes. I find it very difficult to arrive at any conclusion in regard to the comparative depth of the cortex in these two brains without more careful study, but in the new-born brain the cells in the calcarine cortex are certainly more numerous and more closely packed than in the same region in the eight months brain.

A frontal section through the midbrain just anterior to the temporal pole and passing through the anterior island shows a band of closely packed neuroblasts passing in a broad sweep from the inferior horn of the lateral ventricle beneath the cross-cut bundles of the internal capsule, around the inferior and external margin of the lenticular nucleus, gradually growing narrower and less distinct as it passes upward and outward (fig. 2). This band of neuroblasts is quite easily seen by the naked eye in well stained sections. Outside this band is a broad pale zone following the same direction and continued upward around the external

border of the lenticular nucleus nearly to the level of the superior horn of the lateral ventricle, separating the lenticular nucleus from the mass of the corona radiata which is contiguous to the cortex and which is thickly strewn with migrating neuroblasts. This pale zone is sharply defined externally by a more or less compact line of neuroblasts which in horizontal sections would

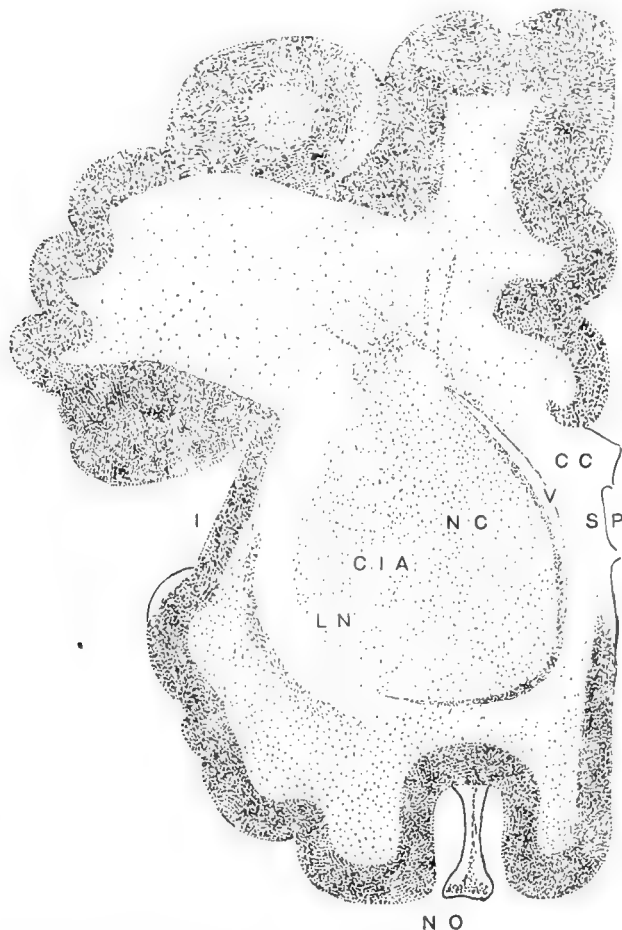


Fig. 2 Transverse section, right hemisphere, of a new-born child, just anterior to the temporal pole. Enlarged $2\times$. *CC.*, corpus callosum; *CIA.*, cross-cut bundles of the anterior limb of the internal capsule; *I.*, island of Reil; *LN.*, lenticular nucleus; *NC.*, caudate nucleus; *SP.*, septum pellucidum.

probably represent a second layer of the 'Übergangschicht.' The wall of the ventricle covering the mesial surface of the caudate nucleus is actively producing neuroblasts, while the opposite wall of the ventricle is much less active although not in a state of rest. In this brain the blood vessels are much engorged (probably due to asphyxiation) and many of those in the direct track

of the migrating neuroblasts are surrounded by closely packed nuclei. This is quite suggestive of the way in which small objects floating down stream collect temporarily about an obstruction. This was not observed in the eight months brain and is possibly pathological, as all the blood vessels in the new-born brain were engorged.

Thus it appears that in man, even at the period of birth, all the constituent parts of the cerebral cortex are not only *not in situ* but that the birth of new units is still going actively on and that these elements are still moving from their place of origin in the ventricular wall to their ultimate destination in that latest and highest development of the animal organism. The latest authoritative statement as to the development of the human cerebral cortex based upon personal investigation is to be found in the last work of His published in 1904.¹ He says the germinal cells produce both neuroblasts and glia cells, but he also states that here and there we find developing elements where no germinal cells are present and he agrees with Shaper that they must there develop from undifferentiated cells. He also says the more crowded they become the more both neuroblasts and spongioblasts assume the drawn out form (pear-shaped) and they can only be differentiated by the visible connection of the spongioblasts with connective tissue, and the neuroblasts by the connection with a nerve fiber.

Essick² in a recent paper on the development of the pontine and arcuate nuclei describes the migration of cells from the roof of the fourth ventricle and the wall of the lateral recess, passing through the intervening tissues in closely packed streams. This behavior of the new elements seems to closely resemble that observed in the slides obtained from the two brains described in this paper. In the case of the developing cortex, however, the closely packed cells streaming from the matrix maintained this peculiar formation only as far as the 'Übergangschicht' and from here to the cortex the migration was continued in what might be called 'single file,' but it was apparently more direct.

¹ Die Entwicklung des menschlichen Gehirns währendes ersten Monats. Wilhelm His. Leipsig, 1904.

² Amer. Jour. Anat., vol. 13, p. 25.

The question arises, and it is a most important one, whether all these nuclei proceeding from the matrix represent both neuroblasts and spongioblasts or whether they are spongioblasts alone. His considered the distinctive characteristic of the neuroblast in the younger embryo to be the darker stain taken by the cell at the end from which the nerve process arises. But he distinctly states that in the middle of the third month this is no longer to be depended on. He agrees that the cortical layer exclusively, or very nearly exclusively, now holds nerve cells, but in the inner zone of the intervening layer the tangentially directed cell bodies should be considered glia cells.

In the first or external layer of the cortex (the molecular layer of Meynert) the few scattered nuclei present may be safely looked upon as spongioblasts. At least if any neuroblasts are present they are so few in number they may safely be ignored. But between the nuclei here present and the naked nuclei swarming in the different layers of the cortex, even at birth, I can detect no characteristic differences. Some of the nuclei in the broad streams going out from the matrix and in the 'Übergangschicht' have partly developed cell bodies and distinct protoplasmic processes, but the great majority are naked nuclei and correspond in every way to the majority of those already arranged in the cortical layers. Some are doubtless spongioblasts, but it is hardly possible that they represent more than a small percentage. It seems quite evident that many neuroblasts reach the cortex where they arrange themselves in their ultimate position before they have developed any protoplasmic processes or possess any demonstrable cell bodies. His suggests that the protoplasmic process may serve as a locomotor apparatus, but the majority of the nuclei are clearly able to reach their goal without it. He speaks of the route followed by the cells in their migration from the matrix to the cortex as always radial, and in this respect differing from that taken by the neuroblasts of the spinal cord and the medulla oblongata. This is apparently true in the earlier stages and would apply up to the end of the fourth month of foetal life, at which stage he thought the migration was completed. But as the brain develops, the space between the matrix and

the cortex becomes filled with new growths and the nuclei can no longer pursue the purely radial direction.

I have not been able to find any satisfactory explanation of the formation of the 'Übergangschicht.' With the exception of the claustrum, for which it appears to form the anlage, it is only a transitory formation, although distinct traces of it persist at birth and perhaps longer. Preparations from the occipital lobe in the eight months brain have been described. In those preparations (fig. 1) the 'Übergangschicht' is still present as a layer of closely packed nuclei almost completely surrounding the ventricle but separated from the matrix by a pale layer containing only scattered nuclei. Several streams of nuclei lead from the matrix to the 'Übergangschicht', but instead of taking a radial direction they run for some distance along the wall of the ventricle and parallel to it, and then leaving the ventricular wall join with other streams to form this closely packed layer. From the external border of the 'Übergangschicht' its component parts appear to be migrating in a radial direction towards the cortex. Why nuclei destined to take part in the formation of the cerebral cortex should collect in a distinctly formed band which apparently persists through many months of intra-uterine life before proceeding on their further journey is a question even harder to answer than that of the propulsive force which carries them to their appointed destination. His states that the formation of the cortex goes on not only during the entire third but also the greater part of the fourth month. I do not find any statement of his that the process is complete at that time, but it is generally understood that such was his belief. All authorities seem to agree in fixing the time of the completion of the migration of the neuroblasts somewhere about the end of the third or fourth month of foetal life. According to Jackson³ the volume of the central nervous system at the third month is about 7 cc. and at birth 376 cc. In the interval between these periods the volume of the central nervous system has increased more than fifty-three fold and the cerebral cortex has nearly doubled in thickness not-

³ Prenatal growth of the human body. C. M. Jackson. Am. Jour. Anat., vol. 9, 1909.

withstanding the proportional increase in the superficial area due to the development of fissures and convolutions. By this the superficial area must be made more than double that of the plane surface.

In the recently published work by Keibel and Mall,⁴ Streeter states that the "migration is most active during the third month and continues well into the fourth." In the English edition he says "at this period" (end of the fourth month) "the wandering of the cortical neuroblasts is completed." In the German edition the statement 'um dieser Zeit' is not quite so definite. He says further "The ependyma does not appear as active as heretofore although it apparently is still giving off spongioblasts that are to form the neuroglial elements of the white substance. The cortical or pyramidal layer has taken up all its wandering neuroblasts from the deeper layers and is sharply marked off from the subjacent intermediate layer." He assumes that all the new elements given off by the matrix after the end of the fourth month are spongioblasts, although His expressly states it is impossible at this stage to distinguish between spongioblasts and neuroblasts in the primitive granular form. The question may arise here as to whether or not the matrix may not again become active after passing into the so-called resting stage. This might explain why what appears to be its great activity in later stages has been overlooked.

Probably at some period not long after birth the matrix is exhausted and no longer produces new elements. The indications are, that proceeding from the caudal end of the neural tube cerebralwards, the matrix is actively productive of new elements in successive stages; that is, as one segment becomes exhausted and goes into a state of rest the next contiguous segment becomes active, and so on until the last and highest segment takes up the work and gives birth to the cells that form the cerebral cortex. The development of many of these elements goes on rapidly during the migration to the cortex. His has estimated from careful measurements of the thickness of the cortical layer at various

⁴ Human embryology. Keibel and Mall, 1911-1912. Lippincott, Philadelphia, U. S. A.

stages of embryonic life, that the period occupied by the migration of a cell from the matrix to the cortex must be at least half a day. This seems to ignore a possible delay in that 'half way station' the 'Übergangschicht.' But Streeter may be right in what I take to be his assumption, that the 'Übergangschicht' is largely made up of spongioblasts. If in the early stages, during the third and fourth months, a neuroblast can pass from the matrix to the cortex in half a day when the distance through the *Zwischenschicht* is comparatively slight, the time occupied by the migration of an element some months later becomes complicated by distance and intervening obstructions. An indication of greater time occupied in the migration is the greater development of the cell body and processes to be seen in certain migrating cells still in the white matter in the eight months and new-born brain.

It is a very difficult matter, perhaps impossible, to say whether or not any given nerve-cell in the cortex, or elsewhere, is *fully* developed. We find in the cortex of every age cells large and small, and every gradation in size of the cell body between these, to say nothing of numerous granules with no cell body. The cell body and its processes probably develop under the demands of functional activity. No one can say that any cell body has reached the limit of its growth. Comparing the cells in the cortex of the new-born with those in the adult brain I conclude that no cell in the cerebral cortex is fully developed at birth. The increase in volume of a cortical cell during the development of the cell body, nerve fiber and processes varies greatly according to location and function.

It is the belief of the writer that all mental development has an anatomical basis. In a comparative study of the cellular structure of the so-called 'Broca's area'⁵ in the brains of three individuals there was found a very appreciable difference in the thickness of the cortical layers in favor of the left hemisphere. It is almost impossible to say just where this difference lies. The counting of cells in a cortical area is extremely difficult, although

⁵ A contribution to the study of the cerebral cortex in man. Mellus, *Anat. Rec.*, vol. 5, p. 473.

by no means impossible. In many instances the count varies so considerably in contiguous areas of the same convolution, due sometimes to the presence of blood vessels and the doubtful nature of many of the cells that it is difficult to arrive at satisfactory conclusions. As a rule, however, it appears that the deeper cortex has the greater number of cells. We would naturally suppose that a cortex increased in depth by increased functional activity would be due either to increase in the volume of the cells or separation of the cells by reason of an increase in the outgrowing or ingrowing processes, or both together. From careful and prolonged study of the motor area in the human cortex I am convinced that there are great variations in different individuals in the development of the largest elements—the so-called Betz cells.

Much time and study has been expended in the effort to find an anatomical basis for intellectual development in brain weight and in the comparative complexity of the convolutions. Such efforts have so far been without result. Many millions of cells may vary greatly in development and the weight of the brain be inappreciably affected.⁶ Estimates based on a careful count in different cortical areas of the adult human brain place the number of cells in a cubic millimeter of cortical substance at about 100,000. The total area of cortex in a brain weighing 1360 grams is estimated by Donaldson at 2352 sq. cm. On that basis a cortex of 2.5 mm. average depth would contain nearly 6000 million cells while a cortex averaging 3 mm. in depth would contain more than 7000 million cells.

⁶ A note on the significance of the small volume of the nerve cell bodies in the cerebral cortex of man. H. H. Donaldson, *Jour. Comp. Neur.*, vol. 9, 1899.

THE DEVELOPMENT OF THE PROOTIC HEAD SOMITES AND EYE MUSCLES IN CHELYDRA SERPENTINA

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TWENTY-FOUR FIGURES (TEN PLATES)

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INTRODUCTION

Balfour ('78), working on elasmobranch embryos, was the first to observe that the separation of the mesodermic layers which gives rise to the body cavity of the trunk, extends forward into the head, and forms a cavity which later becomes

divided into a number of segments, called by him head cavities. These head cavities, later looked upon as true somites, became, as is well known, of considerable importance in connection with the question of the original segmentation of the vertebrate head.

The bulk of the work on the head cavities has been done on fishes, especially elasmobranch embryos, and a varying number of such cavities has been found in different members of the group, nine being probably the average. The head cavities or somites lying in front of the otic region have furnished the greater interest, as here evidence of segmentation has been effaced to a greater degree than in the opisthotic region, where cranial nerves of the vagus group, well differentiated somites, and the arterial and gill arches have been considered by investigators as strong evidence of metamerism. The scarcity of metameric evidence in the prootic region is associated with the progressive development of the brain and associated sense organs, so that the higher we proceed in the vertebrate series the more obscure become the traces of any metamerism which may have existed in the precursors of the group.

The problem of the head somites in the Amphibia has received little attention, although Scott and Osborn as early as 1879 found that a portion of the coelom was present in the head and became segmented by the development of gill clefts; and Miss Platt ('94) found somites in *Necturus* corresponding to the prootic somites of elasmobranchs. In the Mammalia no head cavities can be said to have been found, although Zimmermann ('99), for a human embryo of 3.4 mm., has described a number of small but clear-cut vesicles which may have been vestiges of such structures. For reptiles and birds on the other hand, cavities or somites corresponding to the first three head somites of elasmobranchs have been established. These cavities or somites accord with the prootic somites of the latter group in that they occupy corresponding positions in the mesoderm, have corresponding nerve relations, and in each case give rise to corresponding muscles of the eye-ball. But whether a particular somite in one class of vertebrates is strictly *homologous* with a particular somite in another group, is of course uncertain.

To morphologists looking to the problem of the segmentation of the vertebrate head, the important question is whether these so-called head somites or cavities represent true somites, comparable to the somites of the trunk and occipital regions, and are therefore marks of a primitive segmentation of the head. The answer to this naturally depends upon each investigator's individual conception of what constitutes a true somite; and, as Filatoff ('07) has pointed out in this connection, the variability of a somite in the head region of higher vertebrates, due to the disturbing influence of a greater development in brain and sense organs, must be taken into consideration. Some forms, for instance, may be so highly specialized that all the characteristics of a typical somite have dropped out. As will be noted in the review of literature, the question has been answered in the affirmative by investigators of the head cavities in the Reptilia. For the Aves, Rex ('05), from extensive studies on this group, believes that these structures are products of the visceral mesoderm, and hence cannot be considered true somites, which are differentiations of the dorsal, or paraxial, mesoderm only.

REVIEW OF LITERATURE

THE HEAD SOMITES

The first observations on the head somites of the Reptilia were made by Van Wijhe ('86). This author, in embryos of *Lacerta*, found what he considered to be the homologue of the first head somite of selachians, in the form of a large sac the wall of which consists of a single layer of cells, lying on each side close to the posterior surface of the optic vesicle. There was no connection between the cavities of these two sacs. Because of their position, their association with the oculomotor nerve, and the later transformation of parts of their walls into the same eye muscles that arise from the first head somite in selachians, Van Wijhe declared them homologous structures.

In regard to a second head somite in reptiles, Van Wijhe makes no mention.

At a place corresponding precisely to the position in which the third head somite of selachians is situated, Van Wijhe found a solid mass of cells formed from indifferent embryonic mesoderm, which grows forward, becomes associated with the abducent nerve, and gives rise to the *Musculus rectus lateralis*. This cell-mass he accordingly calls the homologue of the third head somite of selachians.

Hoffmann ('88), also working on embryos of *Lacerta*, (*L. agilis*), found that at a stage in which the optic vesicles are being formed, the first head somites are rather small cavities, one on each side, the walls of which consist of a single layer of cells. These somites are both elongated medially into processes connecting one with the other in the midline. The cavity of the somite does not continue into the process, the walls here being closely apposed. By further development these somites become greatly enlarged, and are then connected by a cross-canal ('Quer-canal') which, at first narrow, soon becomes extraordinarily wide. The end of the notochord lies in close contact with the posterior wall of the canal. Later the canal disappears and out of the walls of the somite are developed those eye muscles which are innervated by the *nervus oculomotorius*.¹

Lying above the first gill cleft and just below the ganglion of the *N. trigeminus*, or exactly in the position where the second head somite of selachians is situated, Hoffmann found a cell-mass conspicuous on account of the peculiar arrangement of its elements. The cells on the periphery are plainly arranged as an epithelium, lie in a single layer, and enclose a rather indistinct cavity, "so that quite evidently we have to look upon this cell-mass as the homologue of the second head somite."

A short distance posterior to the second head somite, but somewhat further medially, Hoffmann found in the same developmental stage, and on each side, two smaller separate and distinct cell masses, in which also the cells are more or less epithelial in their arrangement, and show traces of a small enclosed cavity.

¹ The muscles supplied by the oculomotor, trochlear, and abducent nerves respectively, will be frequently referred to as the 'oculomotor,' 'trochlear' and 'abducent muscles.'

Hoffmann was at a loss as to how to interpret these two masses, but he stated that it was conceivable that the anterior one corresponded to the third head somite of selachians, the posterior mass to the fourth. As opposed to this he found that the latter did not occupy a position above the second gill pouch and below the auditory vesicle, as does the fourth head somite in selachians, but that it lies above the first gill cleft. Furthermore, in selachians the *M. rectus lateralis* is developed from the third head somite and is innervated by the *N. abducens*. In lizards another muscle is also innervated by this nerve, namely the *M. retractor oculi*. This muscle is not found in selachians. It is probable therefore, according to Hoffmann, that both cell-masses belong to the third head somite and that the anterior mass gives rise to the *M. rectus lateralis* and the posterior mass produces the *M. retractor oculi*. This he was unable to verify for lack of material.

The head somites of *Anguis fragilis* were investigated by Oppel ('90). The youngest embryo studied by him was one of 11 segments. At this stage, at the place where Hoffmann found the first head somites of *Lacerta*, Oppel describes his observations in effect as follows: From the point where the anterior, blind end of the foregut abuts against the floor of the forebrain the mesoderm extends from the midline laterally into the head, on both sides. In front of this point there is no mesoderm. A part of the laterally extending mesoderm is noticeable as being sharply differentiated from the rest. This part grows out laterally from the midline, gradually broadening, and extends anteriorly toward the eye. It has the form of two wings attached at a common point. These mesodermic wings lie behind the optic vesicles, only slightly separated from them, and, curving around them laterally and ventrally, they extend still further forward. The connecting-bridge ('Verbindungs-brücke') of these two wing-like structures, which at the same time forms the point at which the chorda and gut-wall meet, has a posterior protruding thickening or process, in which the end of the chorda disappears. Oppel calls this structure uniting the organs mentioned the prechordal plate ('Praechordalplatte'). The foregut touches

the prechordal plate below. The mesodermal wings differ from the surrounding head mesoderm in that their cells are more densely packed. In some sections the cells in the lateral region of the wings are otherwise arranged. There appears here a small cavity, around which the cells are radially placed. Such a structure, according to Oppel, is a characteristic somite.

The cell-stalk which extends from the somite to the midline, Oppel calls the 'Stiel,' and its length, according to him, permits the somite to lie at some distance laterally behind the optic vesicle. The somite portion differs from the corresponding somite found by Hoffmann in *Lacerta*, in that it is sharply marked off from the 'Stiel' or connecting-stalk.

A short distance anterior to the auditory vesicle, at the side of the hindbrain, Oppel found the homologue of the third head somite of selachians as a mass of cells arranged radially about a small cavity. On the cranial border of this somite, seemingly growing out from it, he observed a smaller rather indefinite cell-mass, which he interprets as corresponding to the anterior of the two somitic structures found at this place in *Lacerta* by Hoffmann. The structures here were not separate and distinct from each other as in *Lacerta*, and Oppel was unable to add to the suggestion as to their significance.

Regarding a second head somite in *Anguis*, Oppel is less certain. In an embryo of eleven segments, however, a short distance caudad of the first head somite and somewhat nearer the midline, he found a small structure which answers the requirements of a typical somite. Oppel's figure shows it in section as having a well defined epithelial wall, one cell deep, enclosing a small but distinct lumen. It has no connection with any other structure, and a similar body occurs also on the other side. In an embryo of thirteen segments he found only a small heap of cells at this place, and in older specimens no further trace of it was found. He could establish no connection between this somite-like body and the later appearing *M. obliquus superior*.

The *Lacertilia* have been investigated also by Corning ('00). His observations were made upon embryos of *Lacerta muralis* and *L. viridis*, two forms representing essentially like conditions.

In an embryo of the latter species of 9 segments, two wing-like cell-masses, similar to those of *Anguis fragilis*, partly enclose the ventral wall of the brain tube; but no structure having somite characteristics is present. The 'Stiel' or connecting-stalk of the somite, in the midline, is connected with the entoderm of the foregut, but Corning makes no mention of a prechordal plate. Later a cavity appears in the lateral part of the wing-like cell-mass, which gradually expands into a large sac with walls of cubical or even flat cells. The cavity has continued through the cellular 'Stiel' so that a very wide canal now connects the right and left somites.

Corning evidently found no structure corresponding to the second head somite of Oppel and Hoffmann, and treats of the development of the *M. obliquus superior* in connection with the gill-arch musculature, as it arises, according to him, from the dorsal portion of the trigeminal muscle anlage which grows out anteriorly above the eyeball.

For the third head somite this author recognized a structure which he states agrees in every respect with the third head somite of *Anguis*, and later gives rise to the muscles innervated by the abducent nerve. This somite, he says, is difficult to locate in younger stages, but later is easily found as a cell-mass lying close to the lateral side of the internal carotid artery and somewhat medial to the trigeminal ganglion.

For the *Chelonia*, the only work on the head somites known to the writer is Filatoff's article ('07) on *Emys lutaria*. According to this author, the first head somite of *Emys* is developed from a mass of cells which grows out laterally from the thickened dorsal wall of the anterior end of the foregut. This thickened part of the gut-wall forms at that stage the common origin of both notochord and first head somite. The middle portion of this thickening then differentiates into the chorda, the lateral portions grow outward and give rise to the first head somites. In an embryo of 18 segments the laterally lying first head somites are still connected in the midline by the cell-mass which pushes out from the intestinal wall, which Filatoff now calls the 'Zwischenplatte,' and which corresponds to the 'Praechordalplatte' of

Oppel. The end of the chorda approaches this closely, but is separated from it by an insignificant cell-mass which later becomes more sharply differentiated from both the 'Zwischenplatte' and the chorda, but eventually degenerates into mesenchyma. When fully developed the first head somites in *Emys* are large, thin-walled 'sacs' connected with each other by a narrow canal resulting from the 'Zwischenplatte.'

The second and third head somites were found differentiated in an embryo in which spiracular and first gill clefts had appeared. The second somite lies just below the developing N. trigeminus; it has a lumen and its upper or dorsal wall, especially, is formed by a distinct layer of close-set cells. The third head somite is represented by a heap of cells lying between the second somite and the auditory vesicle, and above the spiracular cleft. In this stage it possesses neither lumen nor the characteristic radiation of cells. Later, however, a rather indistinct radiation appears, and this is the only character, according to Filatoff, which gives this structure claim to being a true somite. A cavity is at no time developed. A compound nature of the somite such as described for the *Lacertilia*, was not observed in *Emys*.

THE EYE MUSCLES

Investigations of the development of the eye muscles in *Reptilia* have been fragmentary. The works of Corning ('00) and Filatoff ('07) contain the most complete accounts.

According to Corning the oculomotor muscles arise at definite places on the wall of the first head somite. These are chiefly the dorsal and ventral regions, while the lateral region, and the antero-medial wall which is directed towards the optic cup, take no part in the formation of the eye muscles. From the first mentioned parts muscle-forming cells grow out forming muscle 'buds,' and at the same time out-pocketings or folds of the wall occur, but to no great extent. The muscle buds or anlagen thus formed grow out dorsally and ventrally, and then take an anterior and lateral direction towards the eye-ball. The ventral outgrowth takes the lead; it is bifurcated at its anterior end,

and Corning compares these two divisions with Hoffmann's findings for *Acanthias*, and would call them respectively the *Mm. obliquus inferior* and *rectus inferior*. Both are connected with each other for some distance from their point of origin on the somite wall.

The dorsal anlage has not, at this stage, advanced very far and Corning states in regard to it that he was able to establish only that it divides into parts to which the upper branch of the *N. oculomotorius* is given off, and that these parts give rise to the *M. rectus superior* and probably to part of the *M. rectus medialis*. The development of these muscles was followed no further by Corning.

As before mentioned, the *M. obliquus superior*, according to this author, arises from the dorsal part of the trigeminal or maxillo-mandibular muscle anlage. On plate 6, figure 33, he pictures the *M. obliquus superior* as an uninterrupted dorsal extension of the trigeminal muscle-mass, ending a short distance above the ophthalmic division of the trigeminal nerve. No structure answering to the second head somite of other authors is thus recognized.

The abducent muscles are derived from the earlier mentioned third head somite. Corning does not associate the two muscles of this group with any two divisions of the third head somite, and does not give figures of the last named structure, because, he states, it agrees in every respect with the figure presented by Oppel for *Anguis fragilis*. From the conditions found in a late embryonic stage of *L. vivipara*, however, he remarks that, for the musculature innervated by the abducent nerve, one has to distinguish between two origins: a posterior one, from which proceeds the greater part of the *M. retractor oculi*; and an anterior one, from which the *M. rectus lateralis* arises. This is because he finds that part of the abducent muscle-mass becomes attached posteriorly to the trabeculae, and part, passing medially between the hypophysis and the trabeculae, becomes attached to the bony plate separating the hypophysis from the oral cavity.

Filatoff's observations on the oculomotor muscles agree with Corning's, but are likewise incomplete. On plate 10, figure 28,

he shows a dorsal and a ventral thickening of the wall of the first head somite. From conditions shown by a much older embryo, figure 32, he concludes that the dorsal gives rise to the *M. rectus superior* and that the ventral one is the common anlage of the *Mm. obliquus inferior*, *rectus inferior*, and *rectus medialis*. The *M. obliquus superior* he derives from the dorsal part of the second head somite.

The abducent musculature is treated by him as one mass from the time of its appearance as a single heap of cells representing the third head somite, up to the advanced stage shown in figure 32, when, he states, a 'Zweiteilung' has taken place, with a corresponding forking of the abducent nerve.

DESCRIPTIVE PART: MATERIAL AND METHODS

The following investigation of the head somites and eye muscles in *Chelydra* grew out of a study of the mesodermic somites of the trunk region undertaken at the suggestion of Dr. B. M. Allen, while doing graduate work at the University of Wisconsin, in the summer of 1910. In continuing this study after returning to Minnesota I became interested in the somites of the head and began a more thorough study of them with the following paper as a result.

A considerable part of the work on this problem including the making of the wax models was done during the past summer in the Laboratory of Comparative Anatomy of the Harvard Medical School. The models were made under the guidance of Dr. Frederic T. Lewis, to whom I am indebted for many favors, helpful suggestions and never failing interest. It is also a great pleasure here to acknowledge the kind interest and encouragement of Dr. Minot, who generously placed at my disposal the entire Reptilian series of the Harvard Embryological Collection, which was found invaluable in checking up and verifying a number of uncertain points in my own series, and for making a number of instructive comparisons. To Drs. Minot and Lewis jointly I am indebted for obtaining the services of Mr. William T. Oliver, of Lynn, Massachusetts, by whom all the drawing of the wax

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models were made, and also for valuable criticism and suggestion in the final preparation of the manuscript.

The material used in this study was obtained by the aid of Prof. H. F. Nachtrieb, from the Embryological Supply Station of Mr. Albert Allen, Madison, Wisconsin. All the material had been faultlessly fixed and preserved. The following fixing agents were represented: Tellyesniczky's bichromate-acetic; sublimate-acetic; and Zenker's fluid. The embryos had all been preserved in 80 per cent alcohol.

The specimens prepared for study were stained in toto in Meyer's hemalum for periods varying from twenty-four to thirty-six hours. With the exception of two early series which were not counterstained all the remaining were stained on the slide in eosin. Sagittal sections proved by far the most satisfactory for the study undertaken. But these were in a number of cases supplemented by cross sections of corresponding stages. A number of temporary wax reconstructions were made from time to time, of various structures, to aid in determining their form and relation to other parts.

THE HEAD SOMITES

The youngest Chelydra embryo studied in the preparation of this paper was a 2-mm. specimen with five segments. In the prootic region of the head the dorsal mesoderm has a compact uniform appearance on each side, becoming less dense towards its anterior limits. A careful scrutiny of the district, however, failed to reveal any differentiation of the mesoderm which might indicate a possible somite area. The next older specimen was a 3.5-mm. embryo with ten segments, in which, as the following description will show, the mesoderm of the head presents differentiations, the earliest phases of which undoubtedly may be observed in stages lying between the two here mentioned.

3.5-mm. embryo (10 segments); transverse series: figures 1 to 3

In this embryo the neural tube is still open at the anterior end. Due to the flexure of the tube the plane of section is hori-

zontal to the region in front of the hindbrain. In carefully examining the anterior portion of this series, beginning with the first sections which are dorsal, and proceeding ventrally, there appears a section at a level slightly above the floor of the mid-brain and passing through both optic evaginations, in which a group of closely packed cells is seen lying in the mesoderm at the side of the neural tube, opposite the constriction between the diencephalon and the mesencephalon. Following this cell-group four sections further ventrally it appears as a well-defined, though rather small, structure in which the cells are arranged radially about a central point; their nuclei are more deeply stained than those in the surrounding mesoderm and lie toward the periphery. In some parts of the structure there seem to be two or three irregular layers of cells, in other parts but one. In the next section (fig. 1) there appears what seems to be a narrow slit-like cavity which can be traced through only two sections. This feature can be made out only with high power (about 300 diameters), but the entire body is readily observed with low power (65 to 80 diameters). Four sections further ventrally the limit of the structure is reached. It thus extends through a total of six 8-micron sections. Frequent mitotic figures occur throughout.

Separated from this structure by not more than two sections, a second group of cells appears, smaller than the first, but showing in two consecutive sections a similar radial arrangement of the nuclei about a central clearer protoplasmic area, with doubtful traces of a lumen. Beyond this group, which extends through four sections, no further differentiations in the mesoderm can be seen in this region.

Through the hind-brain region, the plane of section falls at right angles to the neural tube. The notochord follows the flexure of the tube and is sharply bent so that its anterior end is seen in horizontal section, lying slightly separated from the ventral brain wall. On each side, near the ventro-lateral wall of the hind-brain and at the level of the chordal flexure, a sharply differentiated body appears in the dorsal mesoderm, which resembles a typical somite (fig. 2). Each of the bodies consists of a

layer of tall cells, apparently two or three deep, arranged about a well formed cavity. The one on the left side is the larger and better developed of the two, but in actual size is smaller than the dorsal member of the more anterior group. The cavity can be traced through seven sections. The entire body with its lumen is slightly compressed laterally, so that its dorso-ventral diameter is greater than the latero-medial. Its long axis is parallel to the long axis of the embryo, agreeing in this respect with the two components of the group first described. A short distance caudad the auditory plate appears, and no further mesodermic structures are seen till the first opisthotic somite is encountered just posterior to the auditory plate.

Turning our attention now to the most anterior region of the head mesoderm, in the same section represented by figure 2 there may be observed an elongated area on each side of the forebrain vesicle, which is marked off from the adjacent mesoderm only by the closely packed condition of its cells (S 1). Each mass extends from near the median line antero-laterally, following the border of the fore-brain wall. Followed out, these cell-heaps expand somewhat anteriorly and laterally, and a section passing through the antero-dorsal wall of the foregut shows a rather short bridge of cells connecting the right mass with the left, producing a dumb-bell shape. At its middle this connecting bridge is seen to form a part of a mass of cells pushing out from the anterior dorsal wall of the foregut (fig. 3). The anterior end of the notochord enters this outgrowing cell-mass from the posterior side and becomes indistinguishably fused with it. The two laterally growing cell-masses, therefore, arise from a median mass which pushes out from the entoderm of the anterior dorsal wall of the foregut.

From the position and relations of the three sets of structures here described, it is clear that they represent the three prootic head somites described by Filatoff for *Emys lutaria*, and by other authors for a number of representatives of the *Lacertilia*. From anterior to posterior they are designated the first, second, and third head cavities or somites, or the premandibular, mandibular, and hyoid somites (S 1, S 2, S 3).

The two lateral cell-masses, representing the first head somites, accord perfectly with Filatoff's account of the early stages of these somites in *Emys*. No cavities are as yet to be found on either side. The cells have no definite arrangement nor are they individually distinguishable from ordinary mesenchymal elements. Mitotic figures are numerous and it is evident that the mass is in active proliferation, growth in size taking place both by addition from the wall of the foregut and by cell division within the mass itself. A carefully made wax reconstruction of the region involved shows the first head somites as two quite similar, somewhat irregular masses, which press out on each side from the narrow space enclosed between the floor of the fore-brain vesicle and the wall of the foregut. In each, the large anterior portion narrows somewhat postero-medially towards its origin in the outpushing entodermal cells of the digestive tube. The upper sides of the two somites form a trough-like hollow, closely surrounding the floor and sides of the prosencephalon. Anteriorly they reach to the tip of the neural tube.

The second and third head somites in *Chelydra* lie in a portion of the head mesoderm which is a direct continuation forward of the dorsal mesoderm of the trunk and occipital regions. They answer, according to Filatoff, Dohrn's² requirements for a true somite in that their cells have a typical radial arrangement. Filatoff points out, however, that Dohrn's definition is inadequate in that it does not allow for variations in the form of head somites in the ascending zoological series, due to the modified conditions of their development, and resulting perhaps in complete obliteration of their cavities and a disarrangement of the typical, radial cell order. In *Chelydra* the second head somite shows the very marked radial cell order, but a clear cut lumen cannot, at this stage at least, be claimed for it. It is probable that if a sufficient number of embryos of approximately the same developmental stage were examined, a lumen would be found to exist. In the present investigation but one other embryo of

²Dohrn, a. Studien zur Urgeschichte des Wirbelthierkörpers. Mittheil. aus der Zool. Station zu Neapel. Bd. 15.

precisely the same number of segments was available, and in exactly the same position a cell-group surrounding a small but distinct lumen was found in one section, but as the sections adjacent had been partly lost or mutilated, nothing further could be learned. In the embryo described, the opposite side (the left) presented less advanced development. No such radial cell structure was present, but a rather inconspicuous mass of cells indicated what undoubtedly would have resulted in a corresponding structure. In regard to the second smaller component of the second head somite, it is doubtful whether it is constant, or has any other significance than that it indicates a tendency of the second head somite to split up,—a step towards the diffused condition of this area in birds, as found by Rex. The present phase of the somite is evidently maintained for only a brief period, for in the embryo with ten segments, it was still undeveloped on the left side, and, as will subsequently appear, relatively large cavities soon take its place, which little suggest an earlier somite form.

The third head somite of *Chelydra* differs markedly from the corresponding somite in *Emys* in that it possesses a very clear radial cell structure and a well defined conspicuous cavity. In *Chelydra*, therefore, this somite approaches closely the typical somite form.

4-mm. embryo (13 segments); sagittal series: figures 4 to 7

Distinct advance is apparent in this stage. Cavities have appeared in the first head somite. In the preceding stage, it will be recalled, the anterior end of the notochord disappeared in the thickened dorsal wall of the foregut, from which the cell-masses constituting the first head somites grew out laterally. At the point of cell outgrowth there now lies a median oval thick-walled epithelial body, with narrow central cavity, closely wedged in between the infundibular region and the dorsal anterior wall of the foregut. Its anterior end abuts ventrally against the ectoderm where the invagination of the hypophysis later appears (fig. 4). This structure is the 'Praechordalplatte' of Oppel or

'Zwischenplatte' of Filatoff. The notochord enters its posterior wall, making it appear as though its tip were expanded into the thick-walled prechordal plate.

Owing to the increasing size of the fore-brain the first head somites have been pushed back so as to lie nearly at right angles to the notochord. They are still much flattened in the antero-posterior direction. On the right of the embryo the somite is attached to the anterior end of the prechordal plate by a narrow stalk of cells proceeding from its ventro-medial surface; the somite of the opposite side presents a similar short stalk, but is completely separated from the prechordal plate. In sagittal sections passing through the middle of these somites, each appears as a short crescent, convex posteriorly. There is a relatively large cavity in the dorsal horn, which, followed laterally, branches into two narrower cavities. From the latero-posterior side of the somite a rather slender cellular process extends a short distance caudo-ventrad into a denser portion of the mesoderm of the mandibular arch, which is directly continuous ventrad with the pericardial mesoderm. A cavity is present in the distal end of this process. At this point it will be seen later that the first head somite becomes closely associated with a number of smaller cavities and cell-clusters appearing in the condensed mesoderm area of the mandibular arch, which give rise to the maxillo-mandibular musculature.

The second head somite has undergone a marked change. In exactly the same position occupied by the two somite-like structures of the foregoing stage there here appears a very large, somewhat globular vesicle with a smaller, roughly oval, vesicular appendage on its antero-dorsal wall (fig. 5). The cavities of the two are not continuous. Their respective positions do not point to a formation from two such components as found in the earlier embryos. Individually the cells of the wall do not differ from the surrounding mesenchymal cells, yet, when the structure is viewed as a whole, their close order around the sharply limited lumen, and the numerous deeper-staining nuclei readily distinguish this cavity or somite from other mesenchymal spaces. In many places the wall is distinctly drawn away from the sur-

rounding mesenchyma, and delicate cytoplasmic processes bridge the intervening spaces.

The third head somite also presents marked differences. Instead of the single cavity of the 3.5-mm. stage there lies in its place a compound structure having the appearance of a somite which is about to be divided by a circular constriction into two parts (fig. 6). Each part has a relatively large and distinct lumen, separated entirely from that of the other by a rather thin partition of cells pushed inward from the wall of the somite. The N. trigeminus passes obliquely outward in close proximity to the dorsal wall of the anterior division.

A closer examination of the two parts of this somite shows a number of differences between them. The two are of unequal size. In the posterior division the wall is thicker, and its cells are arranged as a well-defined epithelium about the lumen. This part of the somite also lies further mediad. On the anterior side its wall becomes noticeably thinner, beyond the constriction, as it there passes directly into the wall of the anterior division. The cavity of the latter is somewhat larger. This latter portion of the somite is in the first stages of expansion into the so-called 'cavity' phase. Its wall has come into contact and fused with the median wall of the second head somite, and a narrow canal leads from one into the other. This close association of the second and third head somites is the direct result of the expansion of the two structures, especially of the former.

In the present stage it can be seen that a portion of the mesoderm, extending from just below the outer edge of the second head somite down to where it passes over into the pericardium, has become more densely packed; and immediately ventrad of this somite and adjacent to the ventro-lateral diverticulum of the first head cavity, small clusters of mesodermal cells appear, varying in size, in part solid, and in part enclosing cavities. This entire differentiated area forms the anlage of the musculature of the mandibular arch.

The opposite side of the embryo shows conditions essentially similar, but the second head cavity is more irregular and is flattened somewhat in the antero-posterior direction. It has two

detached vesicles on its postero-dorsal wall. There is no connection on this side between the cavities of the second and third head somites, though their walls are closely approximated. Figure 7 is taken from a second embryo of the same age, and it shows the transition stage between the third head somite of the 4-mm. embryo and that of a 5-mm. specimen presently to be described. It shows clearly the transformation of a typical somite into a large thin-walled vesicle such as, in all head somites, forms the most striking phase.

4.5-mm. embryo; transverse series: figures 8 and 9

A number of sections of this series in the region of the second and third head somites were broken in mounting, and thus rendered unreliable for observation on those structures; but the region of the first head somites is well preserved, and here considerable advances over the preceding stage have taken place. The dorsal parts of these two somites have expanded into large thin-walled cavities, triangular in section, with their bases in close proximity to the optic vesicles and their apices approaching each other near the midline. Further ventrally the large cavity ends, and as seen in sections passing through the prechordal plate, the structure becomes more irregular and consists of a dense cell-mass in which a number of larger and smaller cavities are forming and coalescing, whereby the main cavity becomes gradually extended ventrally and medially into the connecting-stalk (fig. 8). On one side only, the left, is the somite connected with the prechordal plate (fig. 9). The plate itself shows decided change. It is reduced in actual size, its cavity is larger, and the walls are thinner and looser. On one side the cells of its wall continue into a short, more or less tubular stalk, the wall of which then expands laterally into the head somite. There is no connection as yet between the lumen of the prechordal plate and the cavities in the stalk. On the opposite side the connecting-stalk ends freely a short distance laterad of the prechordal plate.

The short flexed portion of the notochord next to the prechordal plate is peculiar in that it is more slender than the

following posterior portion, and is somewhat wavy in its course, giving the impression that it is preparing to disintegrate. Such, however, is not the case at this time. It is still attached to the prechordal plate. In figure 8 the group of cells near the prechordal plate is a part of this section of the chorda.

*5-mm. embryo (20 segments); sagittal series; reconstruction:
figure 19*

On the right side of this embryo the first head somite is a large smooth-walled vesicle, flattened somewhat in an antero-posterior direction, and tapering rapidly medially where its wall passes into a short thin cell-strand (connecting-stalk), ending close to the ventro-medial side of the prechordal plate. Within the median half of the vesicle a slender cell-band extends from the anterior to the posterior wall, being the only remains of the earlier solid mass of the interior. In the ventro-lateral wall of the somite the hollow process of the younger stage is represented by a prominent diverticulum (*dv.*), which tapers down into a short loose strand and becomes lost in the more extensive and conspicuous cell-clusters in the mesenchyma which give rise to the maxillo-mandibular musculature.

The left somite is essentially like the right but somewhat more expanded. It is also connected, through its stalk, with the prechordal plate. A solid portion of the stalk next to the latter structure shuts off communication between their cavities.

Measurements of the prechordal plate at this time show that it is very considerably reduced, as compared with the 4-mm. stage, in all its dimensions. This reduction is the result of the drawing in of its cells into the somite wall by way of the median stalk.

The second head somite of this stage lies somewhat laterad, between the ophthalmic and maxillo-mandibular divisions of the N. trigeminus. Its vesicles here reach their maximum size. As seen in the model the right somite consists of two main lobes or vesicles: a larger dorsal lobe, and a smaller ventral one. The cavities of the two connect by a narrow canal. The dorsal lobe

is itself divided into two nearly equal lobes by an indentation on its lateral side. The ventral vesicle is intimately associated with the muscle anlage of the mandibular arch, in the same way as the diverticulum of the first head somite.

The muscle anlage of the mandibular arch is here differentiated into an irregular, rather extensive dorsal part, and an elongate narrow ventral portion leading down through the arch, in a ventro-medial direction, as far as the pericardium. This ventral portion is now a very well differentiated district in the mesenchyma, and its cells are deeply stained and densely packed; but here and there throughout its extent a number of open spaces occur. The dorsal portion is looser in structure and is less sharply marked off from the surrounding mesenchyma. It seems a common characteristic of the cells of these districts to first form small solid spherules, and then develop cavities, a process quite similar, on a minute scale, to that occurring in a somite.

On the left side of the embryo the second head somite forms a single large cavity, the longer axis of which is caudo-ventral. No connection with the cavity of the third head somite can be made out, but its dorso-medial wall is in contact with the anterior vesicular portion of the latter. Its relations to the mandibular arch musculature are the same.

The third head somite, like the second, in this stage reaches the maximum development of its cavity-phase, but it is a smaller structure. As a whole this somite is elongated, reaching from a short distance anterior to the facio-acoustic ganglion to the dorso-medial wall of the second head somite. The anterior division is a thin-walled sac which is fused on its ventro-lateral side with the dorso-medial wall of the second somite. A broad canal connects the cavities of the two. In the posterior division of the somite the cells have for the most part lost their former more definite epithelial order, retaining it only on a part of the median wall. They appear greatly increased in number, are less sharply limited at the periphery, and loosely surround a relatively small cavity which opens abruptly into the larger cavity of the anterior division. In form this part of the somite is more elongate than formerly. The anterior division of the

somite lies snugly in the angle formed by the ophthalmic and maxillo-mandibular divisions of the N. trigeminus. The trunk of the maxillo-mandibular division lies close against the outer wall of the somite, between its anterior and posterior divisions.

On the left side conditions are similar. The anterior division of the somite seems somewhat more expanded, and on its ventro-lateral side there is a small detached vesicle lying close against its wall. In the posterior division an irregular cavity remains within the loose cell-mass. Between the two portions of the somite is a loose cellular partition, but no open connection between the enclosed cavities is found.

6-mm. embryo; sagittal series: figures 10 to 12

The first head somite of this embryo differs from that in the preceding specimen chiefly in its more expanded condition. In its lateral portion a horizontal septum gives it a two-chambered appearance. On each side the prominent diverticulum of the preceding stage is represented, reaching down to meet an extension of the maxillo-mandibular muscle-mass. This mass or anlage here presents a very sharply defined form throughout most of its extent, being least definite in its dorsal extension towards the first and second head somites. Its long ventral portion, extending down through the mandibular arch, is a thick-walled tubular mass very suggestive of the visceral portion of the mandibular somite in elasmobranch embryos. Dorsally it divides into two short diverging processes, one directed anteriorly (fig. 10, *pr.*) to meet the diverticulum from the first head somite, the other extending towards the second head somite, becoming irregular and indefinite in its upper portion, where it spreads into a diffused area of small cavities and solid cell-clusters.

The prechordal plate is further reduced in size, having been gradually drawn into the median connecting-stalk of the left somite, with which alone it is connected. On account of the increased expansion of the head cavity, the stalk has become shorter proportionately, and now forms a short, rapidly tapering tube on the inner ventral wall of the somite. The somite cavity extends into its base, but the apical portion is solid and fused

with the prechordal plate. On the opposite side the somite has, as before noted, no connection with the prechordal plate.

The rate at which the prechordal plate becomes incorporated into the stalk of the first head somite seems to vary. In another embryo of apparently precisely the same age, the plate is of considerably larger size, lying as a short transverse tube, with compact epithelial walls, against the end of the chorda. On the right side it tapers into a slender cell-cord which then enlarges into the somite stalk. On the left a broader connection exists. The wall of the prechordal plate continues directly into the tubular stalk of that side, and its lumen is confluent with the cavity of the latter. Thus it is evident that the prechordal plate becomes embodied in the stalks of the first head somites and therefore really forms a part of their walls. It is not equally divided between the two, but may be taken up in greater part by either the right or the left, as the case may be.

The second head somites in two series of the stage here concerned show essentially like conditions, but minor variations appear in each. Figure 11 is chosen from the second of the two series, and here the somite is represented by a larger bilobed vesicle which has a smaller simple one lying against its anterior wall. The bilobed form of the larger vesicle is caused by the deep indentation of its lateral wall. This somite is the simplest of the mandibular somites in the two embryos, since it has the smallest number of lobes and independent cavities. In the others a greater number of cavities occur but they are of smaller size. In all cases, however, the cavities of the second head somite are sufficiently closely grouped so that they may readily be distinguished as a whole from the similar though generally smaller cavities of the mandibular arch.

The third head somite is represented by a large mass of cells occupying most of the space between the trigeminal and facio-acoustic ganglia (fig. 12). The roof of the hyomandibular cleft approaches it closely from below. A large, densely packed mass forms its posterior division, and from the anterior side of this a narrow depressed cell-heap extends forward, closely hugging the ventro-medial side of the maxillo-mandibular division of the

trigeminal nerve, which it barely crosses. This represents the anterior vesicular portion of the somite. Its cells are spindle-shaped, and all lie in a general longitudinal direction. In the posterior portion of the somite the cells are for the most part promiscuously heaped, but they assume a distinct spindle form anteriorly on the outer side, where they pass over into the anterior division.

7-mm. embryo; sagittal series

In this embryo the beginning of the epiphysis is present as a distinct epithelial bud. In general form the first head somite is the same as in the preceding stage, but it is somewhat larger. On one side the narrow distal end of its ventro-lateral diverticulum has fused with the neighboring end of the tubular process from the muscle anlage of the mandibular arch, and only a few loose cells on the inner wall of the latter, separate their cavities. This is the most intimate association between the first head cavity and the cavities of the mandibular arch found in the present study. The other side of the embryo, however, shows relations essentially as in the preceding stage.

The prechordal plate is almost entirely absorbed into the median stalk, in this case the left. It is simply a slender solid cell-cord which rapidly broadens into the stalk. The end of the notochord has severed its connection with the prechordal plate, and is more sharply bent ventrally and backward, so that it touches the plate with its dorsal side.

A second embryo of the same measurements, and of equally advanced development, shows a condition of the prechordal plate which is a further instance of the irregularity of the developmental changes in this structure. In this embryo the plate presents an appearance almost identical with that described for the distinctly younger second series of the 6-mm. stage, i.e., it is a relatively large tubular structure with epithelial walls, lying transversely at the end of the notochord which is closely attached to its posterior wall. In the older embryo the cells of the wall at this point are partially drawn out, as if unable to separate from the end of the notochord. That a part of the prechordal plate may thus remain attached to the chorda and become ab-

sorbed in the mesenchyma, seems probable from the facts in the series which follow.

The second head somite of the 7-mm. stage is of such indistinct and indefinite form that it may easily escape notice. It reaches here the most obscure phase of its development. The more or less conspicuous cavities of earlier stages have collapsed and broken down, and with their disappearance the cells of their walls are with difficulty distinguished from the intruding and intermingling mesenchymal elements. There is a tendency, however, for the cells bounding the cavities to form tiny scattered clusters, and these, together with a few stringlets which are unmistakable portions of the cavity walls, make it possible, guided by its position with respect to the trigeminal ganglion, to determine the approximate extent of the somite district.

Processes similar to the above are taking place in the muscle anlage of the mandibular arch immediately adjacent to the second head somite, but the cavities in this area persist somewhat longer. Nevertheless it is difficult at this stage to draw any sharp line between the two districts.

The third head somites in this embryo have essentially the same appearance as in the 6-mm. series represented by figure 12.

8-mm. embryo; sagittal series

The most noticeable change in the first head somite of this stage is that it has still further expanded so that the ventrolateral diverticulum has been practically obliterated in the process, a shallow broad depression alone remaining. The somite is now a thin-walled sac, flattened in the antero-posterior direction, and tapering rapidly medially into a conical hollow stalk. There is no connection between the somites of the two sides. The prechordal plate is no longer found, having been entirely taken up into the stalk and medial walls of the somite. The notochord is still more sharply bent back so that its tip seems to be in actual contact with the oral entoderm a short distance posterior to the hypophysis. A slight but distinct protrusion of the dorsal entodermal wall occurs at this point. The tip of the chorda bears a small group of cells which clearly differ from

the cells of the notochord itself, and, as before suggested, evidently have been detached from the prechordal plate. The oculomotor nerve has increased in length and now lies close to the posterior wall of the first head cavity.

The second head somite is now reduced to a mass of mesenchymal cells, passing gradually over into the surrounding mesenchyma at the periphery, yet distinguishable as a whole by its denser structure and its noticeably deeper-staining nuclei. A few diminutive cavities may occasionally appear.

The third head somite shows advances over the preceding stage in that it is appreciably larger, more compact in structure and more definite in form. It here consists of a larger cell-heap forming the posterior portion of the somite, from the antero-lateral side of which there extends forward a narrow elongate mass which lies opposite the space between the two divisions of the trigeminal nerve and which represents the anterior portion of the somite. The maxillo-mandibular division of this nerve lies against the middle of the lateral side of the cell-mass so that the position and relations of the two earlier vesicular divisions of the third head somite have practically remained unaltered.

Summary

In the foregoing account it has been observed that during the developmental changes which have taken place thus far in the head somites, the anatomical positions of these structures have been maintained throughout, the changes having been chiefly those of form and size. From the small characteristic structures found in the earliest stages, the second and third head somites, after developing into relatively large vesicular bodies, have been resolved by the breaking down of these, into more or less compact masses of mesenchymal and spindle-shaped cells. These resulting cell-masses are the anlagen of certain muscles of the eye. The second produces the *M. obliquus superior*, and the third the so-called abducent muscles, the *Mm. rectus lateralis* and *retractor oculi*.

The first head somite, differing in its early ontogenetic history from the second and third, has arrived at the maximum expan-

sion of its cavity, and greatly exceeds in size that attained by either of the other two. From the walls of this vesicle the remaining muscles of the eye, the so-called oculomotor group, are destined to arise. Since in the next following stage the first traces of these muscles appear, the further history of the head somites will be followed under the section dealing with the development of the eye muscles. In this process of development the abducent muscle anlage is the first to appear, followed successively by the anlages of the superior oblique muscle and the oculomotor group. For this reason it is thought best to treat the groups in this order.

THE DEVELOPMENT OF THE EYE MUSCLES

9-mm. embryo; sagittal series; reconstruction: figure 20

In this specimen the epiphysis and paraphysis are both prominent outgrowths. The nasociliary branch of the ophthalmic nerve extends forward over the dorso-median surface of the optic cup. The short mandibular nerve (*man. v.*) indents the upper portion of the maxillo-mandibular muscle-mass on its lateral surface but the maxillary nerve has not yet appeared. In the second and third branchial arches condensations of the mesenchyma have appeared, forming the muscle anlages (*mus. 2, 3*) of these arches, and the facial and the glossopharyngeal nerves extend ventrally along their respective borders.

The abducent muscle-mass lies in the horizontal plane, just mediad of the maxillo-mandibular trunk of the N. trigeminus. Viewed from the dorsal side, as seen in the model, it is a stout mass in which the two divisions before described are well shown. The more rounded posterior portion (*retr. oc.*) lies nearer the midline, and the anterior part (*rect. lat.*), represented by an elongate narrower extension from the lateral or outer half of the former, reaches considerably beyond the maxillo-mandibular nerve trunk. In sagittal sections the cephalic end of the anterior division presents a heaped-up disposition of its cells, which differentiates it easily from the rest of this division, in which the spindle-shaped cells have a uniform parallel course. This differ-

entiation becomes more pronounced in the following stages, and is the beginning of a process by which the *M. rectus lateralis*, represented by the greater part of the anterior division, becomes more and more distinct from the *retractor oculi* portion, which is posterior.

The abducent nerve (*n. abd.*) could first be found in this stage. It may be seen as a short slender strand parallel with the ventral wall of the brain, and the first impression is that it lies isolated in the mesenchyma, having connection neither with the brain nor with the abducent muscle-mass. Even under careful examination with high power, its distal end could not be traced to the muscle, but its proximal end was seen to be connected with the brain by a number of very delicate filaments or rootlets, three of which can be clearly followed out. In following stages a greater number of such rootlets occur. By the union of these delicate strands the stouter portion of the abducent nerve is formed, which is readily observed; but before their union the rootlets may easily escape notice.

The muscle-mass of the second head somite shows important changes. Its dorsal portion is moving forward as a strong stream of spindle-shaped cells, which is dorsad of the eye-ball and just laterad of the nasociliary nerve. The remaining ventral portion of the somite is in immediate contact with the maxillo-mandibular muscle-mass, but is easily distinguishable from it. Its cells are spindle-formed and directed ventrally. In sagittal sections the entire mass thus presents two divergent cell-streams: the dorsal forward growing part is the *M. obliquus superior* (*obl. sup.*); the ventral portion (*x*) is of doubtful destiny.

The upper or dorsal portion (*max.*) of the maxillo-mandibular muscle-mass has become more sharply differentiated from the longer ventrally extended portion (*man.*) and is a dense structure lying at right angles to the latter. It is narrowly separated anteriorly from the ventro-lateral wall of the first head somite.

The oculomotor muscles. The right and left premandibular somites in this stage are united by a narrow transverse canal, formed by the union of the somite stalk of each side with the prechordal plate. As noted also in the 8-mm. specimen, the

notochord has a group of cells attached to its tip, which in appearance are quite distinct from its own; and a loose patch of similar cells extends from it to the postero-ventral side of the transverse connecting-canal, in the midline, that is, to that part of the canal which corresponds to the prechordal plate. These cells here also are clearly a part of the latter structure, which has become detached along with the chorda.

From a relatively small area on the extreme ventro-lateral wall of the first head cavity, a rather narrow outgrowth of cells extends forward. The basal portion of the outgrowth receives a slight and irregular extension of the somite cavity; its distal part is simply a mass of spindle-formed cells directed towards the ventral side of the eye-ball. The outgrowth is the anlage of the *M. obliquus inferior* (*obl. inf.*) (See also figure 13.)

The oculomotor nerve has grown further ventrad, and lies in close contact with the mid-posterior wall of the somite. In this region there appears a thickening in the somite wall, but it is so slight that without the aid of the stages following it would be considered of no significance. It is the anlage of the *M. rectus superior*, and is destined shortly to become the largest member of the oculomotor group.

The opposite side of the embryo presents practically identical conditions.

10-mm. embryo (a); sagittal series; reconstruction: figures 21a, 21b; sections: figures 14 to 17

The *abducent muscle-mass* in the 9-mm. embryo already described has the form of a stout rod which is somewhat convex externally. In the 10-mm. specimen the anterior portion of the rod, which gives rise to the *M. rectus lateralis*, has grown out laterally, as seen in figure 21b. This lateral portion is closely wedged into the angle formed by the anterior cerebral vein in front, as it bends downward to enter the *vena capitis medialis*, and the *V. capitis medialis*³ behind, as it ascends and passes

³ The *vena capitis medialis* is the primary vein of the head, situated medial to the cerebral nerves, close to the wall of the brain. Grosser, O. *Die Elemente des Kopfvenensystems der Wirbeltiere*. Verh. der Anat. Gesellschaft, Würzburg, 1907, p. 180.

backward. The latter vein extends along the outer surface of the larger caudally directed part of the muscle-mass, which gives rise to the M. retractor oculi. There can be little doubt that the relation to the veins is a considerable factor in hindering the forward migration of the abducent muscles at this stage, and in directing them outward. In the model the Mm. rectus lateralis and retractor oculi are not sharply differentiated from each other, but in sagittal sections they are well defined, owing to the difference in the direction of their cells, and to the fact that the M. rectus lateralis is more compact. The cells of the M. retractor oculi have migrated forward, extending somewhat dorsally and laterally over the inner end of the M. rectus lateralis, so that in sections the latter muscle, at this end, appears as a heap of cells crowded to a greater or less degree ventrad of the anterior end of the M. retractor oculi (fig. 17). A crossing of the two muscles is thereby begun.

The abducent nerve can now be traced to the M. retractor oculi which it penetrates on the median side of its tapering caudal end.

The M. obliquus superior has greatly increased in length and extends forward in a gentle curve above the dorso-medial surface of the eyeball. Its structure is most compact at about its middle, where it is slightly thicker; it tapers anteriorly and also posteriorly to where it is continuous with the remaining ventral portion of the original somite. This latter part retains its previous relation to the N. trigeminus and to the dorsal mass of the maxillo-mandibular musculature, but is somewhat reduced in extent through its contributions to the M. obliquus superior. Its cell-structure is more open than that of any of the differentiating muscle-masses. The dorso-ventral direction of its distinctly spindle-formed cells suggests that they are moving ventrally to fuse with the maxillary and mandibular musculature; but the latter gives no evidence of having received additions from this source, with the exception, possibly, of the part directly adjacent. In figure 16 it would appear as though a dorsal group of cells of the maxillary portion of the muscle-mass were formed entirely by cells from the mass *x*, but in reality it is but a part

of the now very compact and lobulated dorsal end of the maxillo-mandibular musculature.

The continued connection of the mass *x* with the M. obliquus superior and the great length of the latter, are peculiar features of this stage.

The oculomotor group. The premandibular somites are now well drawn away from the median line, and on the left side only is there a remnant of a former stalk or cross-canal. The two cavities are quite similar in form and show little difference in size, but the right is a trifle more advanced in the development of the muscles. The dorso-lateral wall of each is disintegrating (fig. 21 *b*, *Mes.*). Its cells are passing out into the surrounding mesenchyma and pushing into the somite cavity. The inferior oblique muscle (*obl. inf.*) is large and forms a solid club-shaped mass reaching ventrad well beyond any other portion of the somite. The anlage of the rectus superior (*rect. sup.*) is well established as an extensive solid thickening involving a large portion of the upper half of the posterior wall of the somite. It is roughly quadrangular, being narrow medially and widening laterally, where it is closely covered by the ciliary ganglion. This thickening of the wall takes place by an outward proliferation of cells and no perceptible protrusion into the interior of the somite occurs. The position and relations of the anlages of the right and left sides of the embryo are identical.

In the lateral half of the somite its posterior ventral wall sends out a deep sac-like evagination, the outer side of which is closely apposed to the median side of the upper half of the M. obliquus inferior. The posterior and ventral sides of this sac are thickened. The ophthalmic artery crosses the middle of its posterior wall, and turns cephalad, passing in between the evagination and the M. obliquus inferior, and emerging on the anterior side. The large evaginated portion in question is the common anlage of the Mm. inferior rectus and rectus medialis (*inf. + med. recti*).

The ciliary ganglion (*cil. g.*) at this stage appears as an accumulation of cells which form a thickening of the N. oculomotorius toward its distal end. On the right side the nerve extends a short

distance beyond the ventral border of the ganglion. In older stages the nerve lies near the medial surface of the ganglionic mass from which it gradually becomes separated until it lies free along the medial side of the ganglion; the two are then connected by the so-called short root of the ganglion.

The notochord at this stage is slender, and slightly wavy in its anterior portion, and has a small cluster of cells attached to its tip, as in previously described instances.

10-mm. embryo (b)

Although the measurements for this embryo were the same as for the preceding specimen 'a,' its developmental conditions are somewhat more advanced. The N. trochlearis could here be found for the first time. It extends forward from the brain toward the N. oculomotorius, which it has not yet crossed, and it is still some distance from its muscle. Even after assiduous search, it could not be found in the preceding embryo.

The premandibular somites have suffered extensive changes. On one side the wall has completely broken down and the cavity has disappeared. The only mark of its former existence is an area of sparsely distributed mesenchyma conforming somewhat to the earlier outline of the cavity. On the other side parts of the somite wall are still seen intact in sections cutting the inner end of the M. rectus superior. Laterally the wall becomes more broken down, and the cells grade insensibly into the general mesenchyma. The medial wall of the first head cavities is thus the last to break down, and this is probably due to the fact that this side is least affected by the disturbing influence of the developing eye-muscles.

11-mm. embryo; sagittal series; reconstruction: figure 22

The abducent muscles have made decided progress. They are now well differentiated and distinct muscles situated close to the posterior surface of the ciliary ganglion, medial and ventral to the ophthalmic division of the N. trigeminus. The cells of the M. retractor oculi have continued their movement forward

and downward, so that they now form a triangular mass at the anterior extremity of a slender stalk of cells, as seen in figure 22. This stalk of inflowing cells is the belated caudal end of the muscle, and in it lies imbedded the abducent nerve.

The *M. rectus lateralis* in the former stage occupied a more anterior position than the *M. retractor oculi*, but this is no longer the case since its forward movement has been proportionately less. It is now an elongate, more or less conical mass, taking the same general ventral direction as the *M. retractor oculi*, but it is also directed laterad at an angle of about fifteen degrees from that muscle. Its base abuts against the median side of the broad upper portion of the retractor, and its apex points towards the posterior equatorial region of the eye-ball. It is a much smaller mass than the retractor portion.

The *M. obliquus superior* is here an elongate mass, narrow and cylindrical at its posterior end, but broad anteriorly, where it is flattened in the dorso-ventral direction. It lies close to the surface of the eye-ball, parallel to and slightly mediad of the equator, projecting out beyond the surface of the eye anteriorly. Its posterior end is in close association with the distal end of the *M. rectus superior*. These ends are destined to be extended further forward on the eye-ball before their insertion becomes established. The *N. trochlearis* can be traced to within a short distance of the posterior end of the *M. obliquus superior*, but actual connection cannot be demonstrated.

With respect to the ventral portion of the second head somite, embryo 'b' of the 10-mm. stage and the 11-mm. specimen show practically identical conditions, both in structure and in relation to the developing maxillo-mandibular muscles. In the 11-mm. embryo, however, conditions are somewhat clearer and here small branches from the adjacent veins have penetrated the ventral cell-mass of the second somite. The lateral and medial portions have a more open and decidedly mesenchymal appearance, but towards the middle of the mass the structure becomes more compact, and the spindle-shaped cells with their elongate nuclei resemble muscle-forming tissue. As a whole, the mass appears to be undergoing retrogressive changes.

Oculomotor group. We have seen that the M. obliquus inferior was the first of this group to appear. In the view of the model shown in the figure, this muscle is largely covered by others and to facilitate description it will be taken up last.

The M. rectus superior has now become large and its end of insertion is in close relation with the end of insertion of the M. obliquus superior. Like the last named muscle it is slender at its distal end and increases in diameter towards its end of origin. It extends in a postero-medial direction and crosses the N. oculomotorius at its entrance into the ciliary ganglion, lying in close contact with the anterior surface of the nerve. In crossing it receives a branch from the N. oculomotorius, which enters its dorsal side. It then makes a sharp turn dorsad and again mediad, so that at this stage it lies approximately at right angles to all the other muscles of the eye. The M. rectus superior, it will be noticed, is now developmentally in advance of the other eye-muscles, having most nearly reached its definitive position and relations.

The nasociliary branch of the ophthalmic nerve dips under the M. rectus superior just laterad of the point where it crosses the N. oculomotorius, and takes its course anteriorly, following closely the median surface of the eye-ball.

The Mm. rectus inferior and rectus medialis form one elongate mass extending antero-ventrally over the medial surface of the eye, crossing the optic stalk ventral to its junction with the eye-ball. Just below its middle an oblique constriction divides the mass into two club-shaped portions with their enlarged ends anterior: the dorsal is the M. rectus inferior, and the ventral is the M. rectus medialis. The ophthalmic artery lies against the posterior narrower end of the M. rectus inferior on its medial side. Both muscles have received their branches from the N. oculomotorius. This nerve passes ventrally from the medial border of the ciliary ganglion, curves gently forward, and crosses the attenuated district between the two muscle-masses to penetrate the M. rectus medialis on its medial side. Opposite the thickened distal end of the M. rectus inferior it gives off the branch which enters this muscle on its postero-lateral side.

The M. obliquus inferior at this stage is situated at the outer side of the M. rectus medialis, extending slightly beyond it anteriorly. Its posterior end lies transversely across the slit-like remains of the optic groove. Like the two preceding muscles, it is also stoutly club-shaped, with its broad end anterior. On neither side of the embryo is there as yet any branch from the N. oculomotorius to this muscle, which is the only one of the group that has not received its nerve supply.

Chelydra embryo with carapace of 8.5 mm.

Figure 23 is a reconstruction of the eyeball with its associated muscles and nerves, of a 9-mm. *Chrysemys marginata* belonging to the Harvard Embryological Collection (Series 1085). It represents a stage in development of the eye muscles considerably advanced over that of the preceding stage of *Chelydra*. A reconstruction of these parts of an embryo *Chelydra serpentina* with a carapace length of 8.5 mm. shows a somewhat more advanced condition, but one directly derived from a stage such as shown for *Chrysemys*. As a drawing of this model is not available, Mr. Oliver's excellent illustration of the *Chrysemys* model will adequately serve to elucidate the description for *Chelydra*.

The abducent group. The M. rectus lateralis (*rect. lat.*) has rotated in such a way that it lies approximately transverse to the long axis of the body, in a horizontal plane. It is the most slender of the eye muscles. Its laterally directed end narrows somewhat as it passes outward to become inserted on the posterior surface of the eye-ball in the equatorial region.

The M. retractor oculi (*retr. oc.*) is here a short massive muscle, lying upon the dorsal side of the M. rectus lateralis, and crossing it in an antero-lateral direction, so that an x-shaped figure is formed. As it passes onto the eye-ball, which it reaches somewhat further mediad than the insertion of the M. rectus lateralis, a broad sheet of fibers pushes out from along the postero-ventral border of the muscle and creeps antero-ventrally down over the surface of the eye-ball, towards the junction of the latter and the optic stalk. (In the embryo of *Chrysemys* this

outgrowth has barely started and cannot be seen in the model). It is the further development of this sheet of muscle which results in the very broad median portion of the M. retractor oculi in the adult turtle, where it forms an extensive insertion, partially encircling the optic nerve at its entrance into the eye-ball.

As the two abducent muscles, in approaching their insertion, have so turned as to lie approximately transverse to their former direction, the abducent nerve reaches them at right angles to their long axes. It enters the M. retractor oculi directly on its postero-ventral side. Immediately before penetrating this muscle, it gives off a large branch which follows closely the ventral surface of the M. retractor oculi, on its way to the M. rectus lateralis which it enters from the dorsal side at about the middle of its length.

In *Chelydra* at this stage a slender nerve comes from the trigeminal ganglion at the root of the nasociliary nerve, and penetrates the M. retractor oculi in a direct line with the ciliary ganglion. This evidently is the so-called long root of the ganglion. In the *Chrysemys* embryo in which it has already connected with the ganglion, it arises from the nasociliary nerve more distally (*l.r.*), and passes down between the Mm. rectus superior and retractor oculi, parallel to the N. oculomotorius.

The M. obliquus superior. This muscle (*obl. sup.*) has greatly increased in length. It is fixed at its insertion and is about to become fixed at its origin. Its former position corresponds with its inserted end, from which it extends ventrally and anteriorly, following closely the surface of the eye-ball. As it reaches the nasociliary nerve where this leaves the anterior surface of the eye, it makes a slight bend, passing dorsad of the nerve and continuing medially. In *Chrysemys* the muscle is less advanced and is shorter, but it has the same direction and position with regard to the eye-ball. The N. trochlearis (*n. troc.*) has reached the M. obliquus superior a short distance from its insertion, penetrating its posterior edge.

The oculomotor group. The M. rectus superior (*rect. sup.*) has changed but slightly, having merely straightened somewhat, and grown further forward, and established its insertion. Its

relation to the oculomotor nerve and ciliary ganglion remains the same. Its end of origin lies in close relation with the abducent muscles. In sagittal sections the Mm. rectus superior and retractor oculi are situated dorsally, forming respectively the anterior and posterior members of the group, and the M. rectus lateralis forms the ventral member.

The M. rectus medialis (*rect. med.*) is not soon separated from its twin muscle the M. rectus inferior (*rect. inf.*), and in the Chrysemys embryo there is still a slender connection between them. The M. rectus medialis has worked its way dorsally to become inserted on the antero-medial surface of the eye-ball. In the course of this migration the M. rectus inferior has been drawn anteriorly and lies close against the ventral surface of the optic stalk, below which it has found its insertion. The area of connection of the M. rectus inferior with the M. rectus medialis extends from about the middle of the medial side of the latter to its free end. The condition found in the Chelydra embryo shows that a like relationship of these muscles has existed, but the two muscles are now separate from each other. Evidently the M. rectus medialis, in growing forward toward its insertion, has drawn the free end of the M. rectus inferior further mediad and ventrad.

On the ventral surface of the M. rectus medialis, where it crosses the optic stalk, there is a small, globular, solid cell-mass (*z*) for which I am unable to account, unless it may be an accidentally separated portion of muscle or nerve tissue.

The M. obliquus inferior (*obl. inf.*) has effected its insertion in common with the M. rectus inferior, and has turned about its ventral end in a medial direction, following the change in position of the M. rectus medialis. It has now received its branch from the N. oculomotorius, the nerve entering on its posterior side, a short distance from the insertion.

In Chelydra the Mm. recti medialis and inferior have just become separated, the separation having taken place at their free ends (origins). The M. rectus inferior has been drawn forward in this process, its end of origin having been moved from its proximity to the ciliary ganglion to a position ventral and

anterior to the entrance of the optic stalk into the eye-ball. The inner ends of this muscle and the *M. rectus medialis* are therefore in close proximity. Together with the *M. obliquus inferior* they form a second group, at this stage, such that in sagittal sections the *M. obliquus inferior* is ventral and anterior, the *M. rectus medialis* dorsal and posterior, and the *M. rectus inferior* also dorsal and immediately posterior to the second.

With the exception of the *M. retractor oculi* which is more complex in form than the rest, the eye muscles have virtually attained their definitive positions, the establishing of their origins alone remaining.

The M. retractor oculi of the adult *Chelydra* is of great breadth and length as compared with the other eye muscles, and presents two divisions: a lateral (or external) portion, which arises from the base of the posterior edge of the interorbital septum; and a medial (or internal) portion, the origin of which extends from the origin of the lateral portion, forward, for some distance along the ventral margin of the interorbital septum. The medial portion is developed, as previously remarked, from the sheet-like down-growth of the posterior edge of the original muscle, and it is inserted along an area on the eye-ball partially surrounding the optic nerve. The lateral portion is narrower, and is inserted on the posterior surface of the eye mediad of the insertion of the slender *M. rectus lateralis*. In a lateral view of the eye, the *M. rectus lateralis* may be seen passing up dorsally along the posterior surface of the eye-ball and crossing the external portion of the *M. retractor oculi*.

The conditions in *Chelydra* are essentially as given by Bojanus for *Testudo europaea*, and by Hoffmann for other chelonian representatives.

SUMMARY

The head somites. In embryos of *Chelydra serpentina*, three prootic head somites are developed on each side.

The first head somite arises as a lateral outgrowth of entodermal cells from the antero-dorsal wall of the foregut. The outgrowing cells form a stalked, compact, and more or less irregu-

lar mass on each side, in the interior of which cavities arise. These cavities coalesce, and eventually a large thin-walled vesicle is formed which reaches its maximum size at about the 9-mm. stage. A constant feature of the development of this somite is an extension from its ventro-lateral wall which enters into close temporary association with the developing musculature of the mandibular arch.

The area of outgrowing cells on the wall of the foregut, from which the first head somites are formed, very soon becomes differentiated into a thick-walled epithelial body with slit-like lumen—the so-called prechordal plate—which is connected laterally with the first head somites by a slender solid cell-stalk. The notochord ends anteriorly in the posterior wall of the plate.

As development proceeds, the cavity of the prechordal plate enlarges and its walls become thinner, as some of its cells pass over into the stalks of the somites. In some cases the cavities of the somites push into the stalks and connect with the cavity of the prechordal plate, thus forming a temporary connecting canal between the two somites. But in other instances the stalk of one or the other of the somites may become constricted off from the prechordal plate at a relatively early stage, and whether or not a cross-canal would in such cases be formed is difficult to say without more extensive study. In *Chelydra* the cross-canal, where found, is rather narrow. In some reptilian forms it is very broad. A part of the prechordal plate may remain attached to the end of the chorda and later become lost in the mesenchyma.

The second head somite. The second head somite arises in the dorsal mesoderm at the side of the neural tube, just below and slightly anterior to where the trigeminal ganglion later appears. It probably first appears as a small heap of concentrated mesodermal cells, but the early phase of this somite in *Chelydra* seems less clear than in *Emys* as described by Filatoff. These cells then become arranged in a radial manner about a central point or lumen, and assume the form of a small somite. The somite very soon becomes expanded into a thin-walled vesicle of more or less spherical form which may be accompanied by one

or two much smaller vesicles. These may arise independently from smaller somite-like structures, as described in connection with the 3.5-mm. embryo, or by a budding-off process from the main cavity. The cells forming the wall of the vesicle or vesicles, constitute the somite. The vesicle reaches its maximum expansion at about the 5-mm. stage and may still be accompanied by a small number of minor cavities. A single *main* cavity is always present.

At this stage the ventral side of the somite is in close contact with an area of small cell-clusters and minute cavities, which have arisen in the mesoderm of the mandibular arch, just ventrad of the second head somite. They extend ventro-medially through the arch to the pericardial region, and form the anlage of the maxillo-mandibular musculature. From now on, the somite suffers a collapse of its walls and becomes broken down into a considerable number of smaller cavities, which in turn are reduced, until eventually but a mass of mesenchymal cells remains, scarcely distinguishable from the surrounding mesenchyma.

The third head somite, like the second, arises in the dorsal mesoderm close to the ventro-lateral side of the hind-brain, between the location of the facio-acoustic and trigeminal ganglia, but nearer the latter. In an embryo of 3.5 mm. the third head somite is a well differentiated structure with clear cut lumen and well defined epithelial walls; and it is slightly compressed, so that its principal axis is longitudinal. A little later this somite is subdivided, but at the stage under discussion, painstaking study of the sections, supplemented by a carefully made wax reconstruction failed to reveal any indication of subdivision. The subdivided condition, therefore, is to be considered as secondary, and results from the division of a primarily undivided somite.

The anterior division of the somite rapidly becomes expanded into a rather large vesicle, and for a time it may be connected with the second head cavity through a fusion of a part of their contiguous walls. The posterior portion is of considerably greater mass, has thicker walls and expands but slightly, so that its cavity is relatively small. It is connected for a short time with the cavity of the anterior division, and the walls of the two are

continuous. In this condition (5-mm. stage) the position of the somite is such that the maxillo-mandibular division of the N. trigeminus passes across the mid-lateral wall of the somite and the vesicular anterior part lies in the angle formed by this nerve trunk and the ophthalmic division. The wall of the anterior division of the somite next collapses, and becomes transformed, in a manner similar to that of the second head somite, into a rather narrow mass of spindle-shaped cells which are continuous with the cells of the larger solid mass resulting from proliferation in the posterior division; so that two unequal masses are now formed, representing corresponding divisions of the somite. The anterior mass is the anlage of the M. rectus lateralis, and the posterior of the M. retractor oculi.

In regard to the idea expressed by some authors, that the head cavities form reservoirs for excretory products resulting from the activities of the developing somites, these observations on *Chelydra* can contribute nothing. The technique employed brought out no evidence that there is normally any substance present in these large cavities. In two instances, however, one of the first head somites was found gorged with blood cells. The first case occurred in an embryo *Chrysemys marginata* from the collection of Dr. B. M. Allen; the other in a 10-mm. *Chelydra serpentina* of the writer's series, in which the oculomotor muscles had begun to develop. Compared with the other side where conditions appeared normal, the cavity was more nearly spherical, but it was not appreciably larger, and no disturbance of the developing muscles had resulted. It could not be ascertained positively just where the contents had entered the cavity, but there were some indications that a rupture had occurred in the ophthalmic artery, or perhaps in an adjacent part of the carotid artery itself.

The eye muscles. The first of the eye muscles to be laid down are those arising from the third head somite, or the abducent muscles. But these muscles, however, on account of the greater distance which they have to traverse in reaching their destination, are not the first to attain their adult position; the M. retractor oculi, due to its great complexity, is the last eye muscle to reach its definite position.

When the third head somite has resolved itself into the two unequal but united portions above described, a gradual forward movement occurs. In this the posterior or retractor oculi portion plays the more conspicuous part, taking the lead and pushing its anterior end up along the median side of the M. rectus lateralis. The M. rectus lateralis portion, in the meantime, has become a more compact and better differentiated mass, in intimate association with the antero-ventral end of the M. retractor oculi. When the 11-mm. stage is reached the muscles form two separate and distinct masses of unequal size, lying just ventro-mediad of the ophthalmic division of the N. trigeminus, near the posterior side of the ciliary ganglion. Their principal axes have changed from a horizontal direction to a more nearly dorso-ventral one; and the M. rectus lateralis has also turned laterally towards its insertion on the posterior surface of the eye-ball. Its insertion is established in an embryo with a carapace length of 8.5 mm. and after extending slightly towards its point of origin its final position is reached.

At this stage the posterior side of the M. retractor oculi has given rise to a broad sheet-like down-growth of fibers, the free edge of which grows along the surface of the eye towards the junction of the optic stalk and cup; this part becomes the medial division of this muscle as it occurs in the adult. The muscle then begins to grow posteriorly and medially towards its origin.

The abducent nerve first appeared in the 9-mm. stage. No connection with the abducent muscles can be made out until the 10-mm. stage. Here the nerve has penetrated the retractor portion of the muscle-mass. The abducent nerve arises from the brain by a number of very delicate rootlets, three of which can be counted in the 9-mm. stage; in an 11-mm. embryo six were found. In this stage the M. rectus lateralis has received its nerve supply through a stout branch given off from the N. abducens at the point where it enters the M. retractor oculi.

The M. obliquus superior. This muscle in Chelydra develops in precisely the same way as described by Filatoff for Emys lutaria, and the process accords with that observed in many other vertebrate forms. The muscle grows forward as a stream

of cells from the dorsal portion of the mesenchymal cell-mass which results from the second head somite. It remains connected for a relatively long period with the ventral portion of the cell-mass, and thereby attains great length. It passes forward over the dorsal side of the eye-ball, separates from the ventral part of the somite, and soon after the 11-mm. stage it becomes attached by its posterior end (insertion) at about the mid-dorsal surface of the eye, lying close to the surface of the latter along the equator. From this position the muscle gradually swings medio-ventrally, rotating about its inserted end, till it lies in a meridional direction (8.5-mm. carapace stage) when it continues its growth directly towards its origin, passing just dorsad of the nasociliary nerve.

The N. trochlearis does not reach the M. obliquus superior until a comparatively late period—shortly after the 11-mm. stage. It enters the muscle near its insertion.

Filatoff states than in *Emys* the ventral portion of the second head somite, i.e., the cell-mass resulting from it, becomes a part of the musculature of the mandibular arch. Except for the small part of this mass which is directly adjacent to the musculature of the arch, I am inclined to believe that such is not the case in *Chelydra*. At least the evidence here gives little support to Filatoff's interpretation. The embryo of 8.5-mm. carapace showed no trace of this cell-mass and gave no clue to its probable fate. In younger stages its structure indicates that it is of muscle-forming nature but its progressive development seems interrupted or suppressed. As late as the 11-mm. stage it still remains unchanged in position and form, and its more lightly stained elements and open structure are in marked contrast with the heavily stained and dense masses of the maxillo-mandibular musculature, and with the progressive development characteristic of other muscle-masses. In view of these facts it seems that this part of the second head somite must be interpreted in some other way.

In elasmobranch embryos it was first shown by Miss Platt that from the ventro-median wall of the mandibular somite proper there is formed a muscle 'E,' which early reaches an ad-

vanced development but thereupon degenerates and disappears. Lamb observed this muscle in *Acanthias* embryos, and in his illustrations its position and relations to the *M. obliquus superior* and the muscles of the mandibular arch, are strikingly similar to the conditions in *Chelydra*. It seems to me entirely probable that in the ventral portion of the second head somite of *Chelydra* we have the vestige of a muscle corresponding to this passing muscle appearing in elasmobranchs, the past functional significance of which is unknown.

The oculomotor muscles. The first muscle of this group to appear is the *M. obliquus inferior*. It begins at about the 9-mm. stage, as a somewhat loose outgrowth of cells from a rather small area on the outermost ventral portion of the wall of the first head somite, after the somite has reached its maximum expansion. The outgrowth is not in the nature of a true evagination of the wall, but irregular spaces from the somite cavity extend into its base. It takes a direction anteriorly and slightly ventrad towards the ventral surface of the eye-ball. By the 10-mm. stage it has become a solid club-shaped mass, the narrow proximal end of which still has a loose cellular connection with the somite wall. From this position it slowly moves forward along the medio-ventral surface of the eye-ball, and in a stage represented by an embryo of 8.5-mm. carapace length, the end which has become free from the somite wall becomes attached to the eye-ball, forming the insertion of the muscle; its opposite, anterior end has turned toward its attachment of origin. While it is the first of the oculomotor muscles to appear, it is the last to receive its nerve supply, and is preceded by the *M. rectus superior* in establishing its insertion.

The *M. rectus superior* arises soon after the *M. obliquus inferior*. Its anlage appears as a broad thickening of the posterior wall of the first head somite, near the dorsal side, extending from near its lateral end medially about three-fourths the width of the somite. Its outer posterior side is closely covered by the ciliary ganglion. From the beginning the position of this muscle is such that but a slight change in direction is necessary in reaching its definitive position. It bends antero-dorsally as it ap-

proaches the eye-ball, along the surface of which it extends towards its insertion which it reaches quite simultaneously with the M. obliquus superior, in embryos of about 11 mm. It then gradually straightens out, and grows directly towards its origin. The first branch developed from the oculomotor nerve goes to this muscle and is given off where the nerve crosses its dorsal posterior border to reach the ciliary ganglion.

The Mm. rectus inferior and rectus medialis are the last of the eye muscles to begin their development, and they arise from a common anlage which is formed by a deep out-pocketing from the ventro-lateral wall of the first somite, just mediad of the M. obliquus inferior. This is the condition at the 10-mm. stage. A thickening of the walls of this out-pocketing, especially on the posterior and ventral sides, takes place simultaneously and it becomes transformed into a solid elongate mass. By the 11-mm. stage a constriction has appeared, slightly beyond the middle of this mass, differentiating it into a proximal M. rectus inferior and a distal M. rectus medialis. The distal end of the M. rectus inferior now lies where its insertion later occurs. The distal end of the M. rectus medialis swings gradually dorsalward in the direction of its future point of insertion on the eye-ball, in front of the optic stalk. Its proximal end, which at first is continuous with the distal end of the M. rectus inferior, works up along the medial side of this muscle so that the final separation of the two takes place at their proximal ends, i.e., their ends of origin. This has just occurred in the specimen with an 8.5-mm. carapace. Their insertions have by this time been established, and the muscles are directed toward their points of origin. In the 11-mm. stage each of these muscles has received its branch from the N. oculomotorius.

In regard to the origin of the abducent and oculomotor nerves Filatoff makes the following italicized statements:

Meiner Ansicht nach ist es von grosser Bedeutung, den ersten Zustand des Auftretens der Anlage, welche zwar keinerlei Nerven-elemente enthält, doch in Form und Lage den künftigen Nerv präformiert, d.h. also den Umstand in Auge zu behalten, dass diese Anlage in innigstem und ununterbrochenen Zusammenhange mit der Anlage des Muskels

steht und vom Nervensystem völlig unabhängig auftritt (p. 343, in regard to anlage of N. abducens).

In der Entwicklungsgeschichte des Oculomotorius ist ebenso wie in der Abducens die Tatsache bemerkenswert dass die ursprüngliche Nervenanlage als unmittelbare Fortsetzung der Muskel- oder Somitenanlage dem Nervensystem nur angelagert ist und erst sekundär Beziehungen zu demselben durch die von dorthin in die Anlage hineinwachsenden Nervenfasern gewinnt (p. 363).

The relation of the abducent nerve to the brain and to its muscles, at the time of its first appearance in Chelydra, has already been discussed. Contrary to Filatoff's opinion, the nerve does not arise in continuity with the developing muscles. Connection with the muscles is acquired later. Moreover, the abducent nerve is to be interpreted as formed by the direct outgrowth from the brain of several delicate filaments, which unite to form a bundle that is easily seen. In the early stages of Chelydra it is only on superficial examination that the filaments which connect this bundle with the brain may be overlooked.

Similarly the oculomotor nerve, when first found in the present study, had no connection with the first head somite, but could readily be followed to the brain tube. In the 10-mm. embryo, where the ciliary ganglion appears as an accumulation of cells at the distal end of this nerve, it still has no connection with its muscles. At 11 mm., however, the nerve extends beyond the ventral border of its ganglion and connects with the Mm. recti superior, inferior and medialis. Later it connects with the M. obliquus inferior, and eventually it becomes separated from the ciliary ganglion except for a branch which forms the short root of the ganglion. Filatoff's conclusions, therefore, are not applicable to Chelydra.

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ABBREVIATIONS

<i>aud.</i> , ear	<i>n. oc.</i> , 1, 2, branches of nervus oculomotorius to eye muscles
<i>ac. X</i> , vagus accessory nerve	<i>N. oc.</i> , } nervus oculomotorius
<i>br.</i> , brain	<i>N. III</i> , }
<i>Ch.</i> , notochord	<i>N. troc.</i> , nervus trochlearis
<i>cil. g.</i> , ciliary ganglion	<i>obl. inf.</i> , musculus obliquus inferior
<i>con. st.</i> , connecting-stalk of first head somites	<i>obl. sup.</i> , musculus obliquus superior
<i>div.</i> , diverticulum from ventro-lateral wall of first head somite	<i>op. c.</i> , optic cup
<i>ec.</i> , ectoderm	<i>op. st.</i> , optic stalk
<i>f. ac.</i> , facio-acoustic ganglion	<i>Ophth. art.</i> , ophthalmic artery
<i>F. B.</i> , forebrain	<i>Ophth. V</i> , ophthalmic division of N. trigeminus
<i>fg.</i> , foregut	<i>pre.</i> , prechordal plate
<i>H. B.</i> , hindbrain	<i>rect. lat.</i> , musculus rectus lateralis
<i>hyo. cl.</i> , hyomandibular cleft	<i>rect. inf.</i> , musculus rectus inferior
<i>inf. + med. recti</i> , inferior and medial recti muscles or their anlagen	<i>rect. med.</i> , musculus rectus medialis
<i>l.</i> , lens	<i>rect. sup.</i> , musculus rectus superior
<i>l. r.</i> , long root of ciliary ganglion	<i>retr. oc.</i> , musculus retractor oculi
<i>Man. V.</i> , <i>mb. n.</i> mandibular ramus of N. trigeminus	<i>S1, S2, S3</i> , first, second and third head somites
<i>Max. V.</i> , maxillary ramus of N. trigeminus	<i>S.C.N.</i> , short ciliary nerves
<i>max.-man.</i> , maxillo-mandibular musculature	<i>st.</i> , in model, remains of connecting-stalk of first head somite
<i>mes.</i> , disintegrating portion of wall of first head somite	<i>tri.</i> , nervus trigeminus
<i>mus. 2, mus. 3</i> , muscle anlagen of gill arches	<i>tri. g.</i> , trigeminal ganglion
<i>n. abd. 2</i> , abducent branch to musculus rectus lateralis	<i>x.</i> , ventral cell-mass of second head somite
<i>N. abd.</i> , } nervus abducens	<i>z</i> , cell-mass of doubtful significance and derivation.
<i>N. VI</i> , }	<i>V 2 + 3</i> , maxillo-mandibular trunk of N. trigeminus
<i>na. cil.</i> , nasociliary branch of nervus trigeminus	<i>VII</i> , nervus facialis
	<i>IX</i> , nervus glossopharyngeus
	<i>X</i> , nervus vagus

PLATE 1

EXPLANATION OF FIGURES

- 1 3.5-mm. *Chelydra serpentina* (10 segments). Section passing through head region. $\times 175$.
- 2 Same series. Section passing transversely through hindbrain vesicle. $\times 175$.
- 3 Same series. Section passing transversely through tip of forebrain. $\times 175$.
- 4 4-mm. *Chelydra serpentina* (13 segments). Sagittal section in median plane through prechordal plate. $\times 175$.
- 5 Same series. Sagittal section laterad of neural tube, through the N. trigeminus and second head somite. $\times 175$.

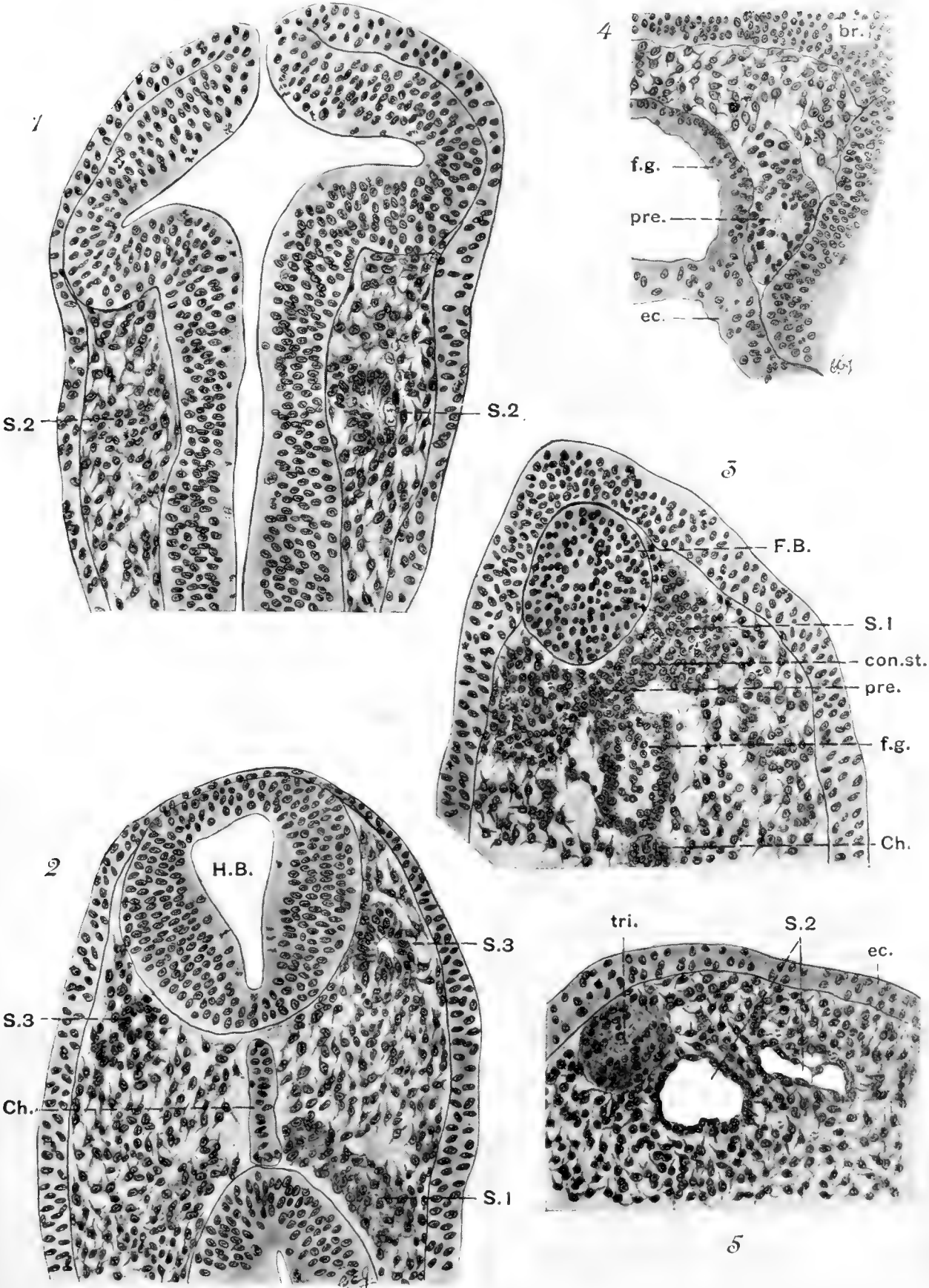


PLATE 2

EXPLANATION OF FIGURES

6 4-mm. Chelydra (13 segments). Sagittal section somewhat further mediad than figure 5, passing through third head somite. $\times 175$.

7 Sagittal section through third head somite of a second embryo Chelydra of 4 mm. $\times 135$.

8 4.5-mm. Chelydra. Transverse section through the forebrain and first head somites. $\times 175$.

9 Section from same series, taken further anteriorly, passing through the antero-dorsal wall of foregut. $\times 175$.

10 6-mm. Chelydra. Sagittal section through the mandibular arch, cutting the outer edge of second head somite and the ventro-lateral diverticulum of first head somite. $\times 175$.

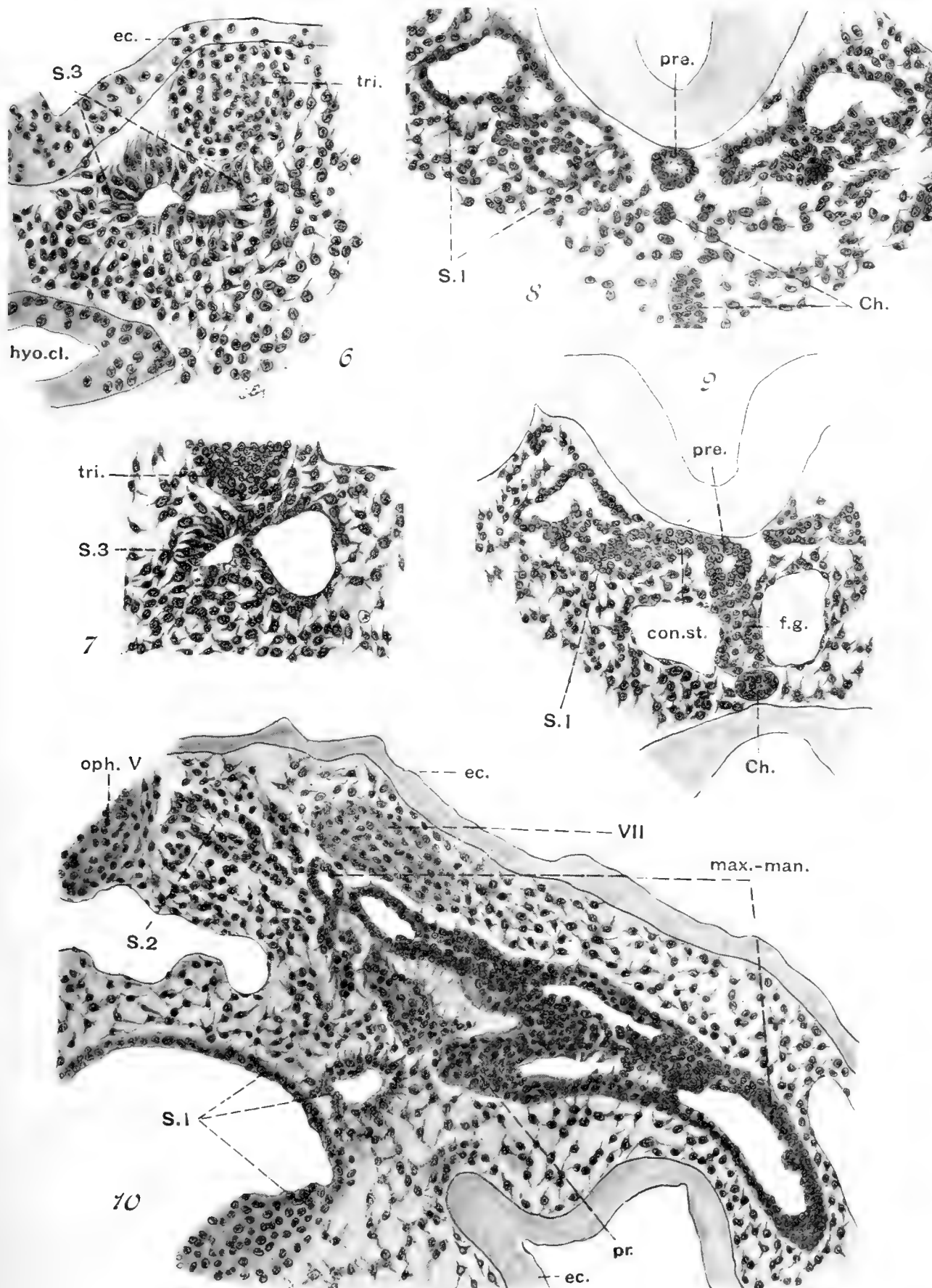


PLATE 3

EXPLANATION OF FIGURES

11 6-mm. Chelydra (second series). Sagittal section through second head somite and outer portion of abducent muscle-mass. $\times 175$.

12 Section from same series somewhat further mediad, passing through the two divisions of the abducent muscle-mass. $\times 175$.

13 9-mm. Chelydra. Sagittal section passing through outer wall of first head somite, showing the M. obliquus inferior. $\times 135$.

14 10-mm. Chelydra. Sagittal section through middle of first head somite and ganglion ciliare. $\times 135$.

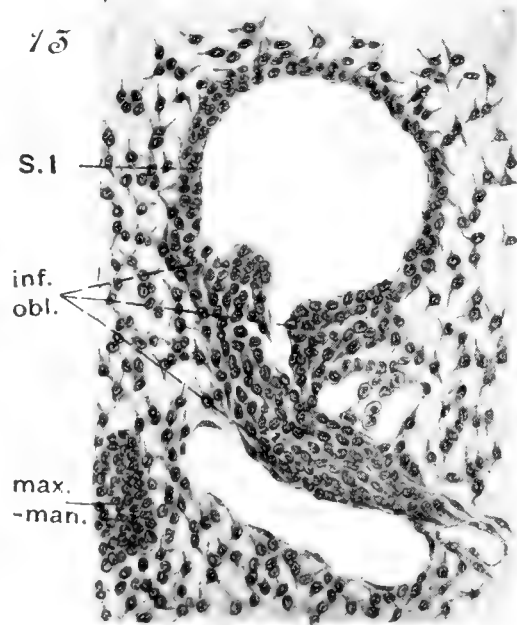
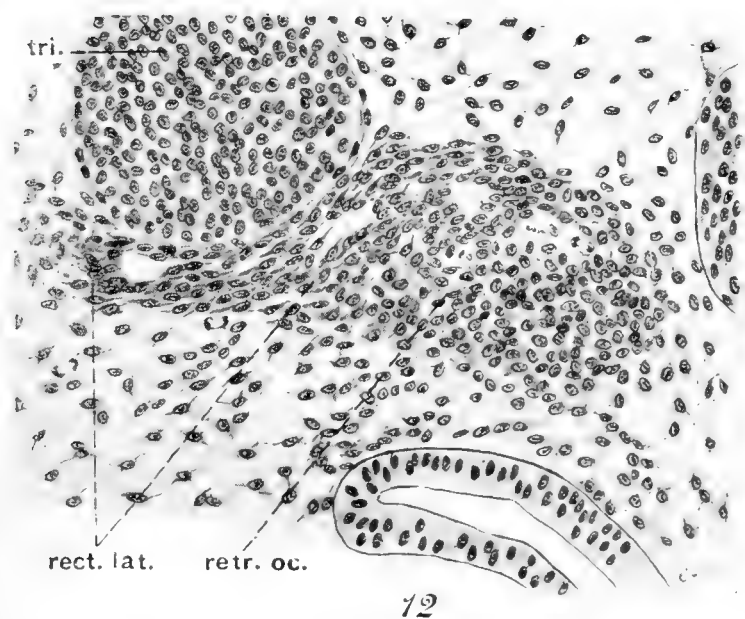
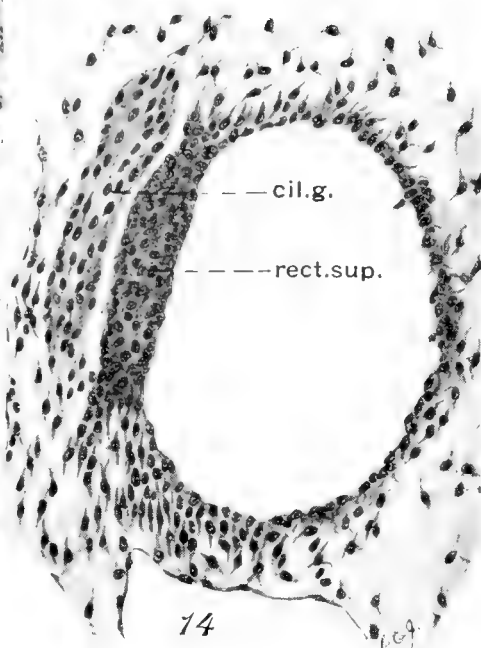
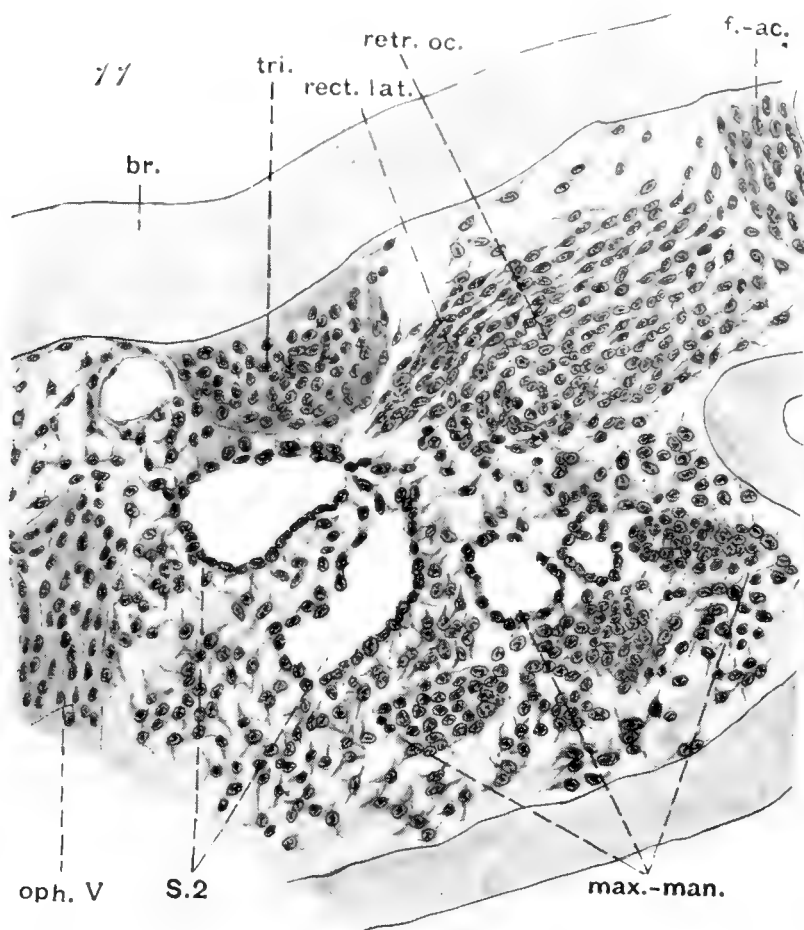


PLATE 4

EXPLANATION OF FIGURES

15 10-mm. Chelydra (same series as fig. 14). Sagittal section cutting lower edge of ganglion ciliare and the outer disintegrating wall of the first head somite. $\times 135$.

16 Same series. Section passing through the eye and the mandibular division of the trigeminal nerve. $\times 70$.

17, 18 10-mm. and 11-mm. embryos respectively, showing the relations of the Mm. rectus lateralis and retractor oculi. $\times 135$.

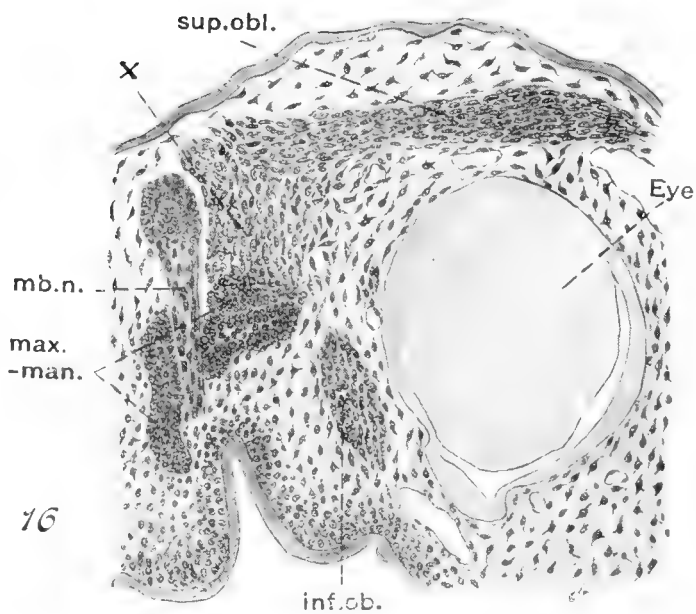
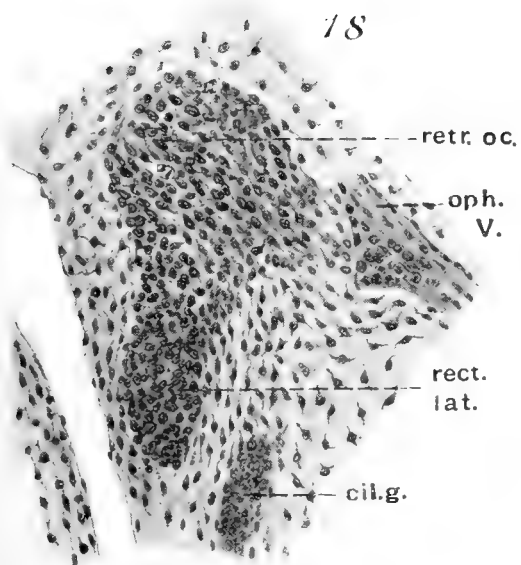
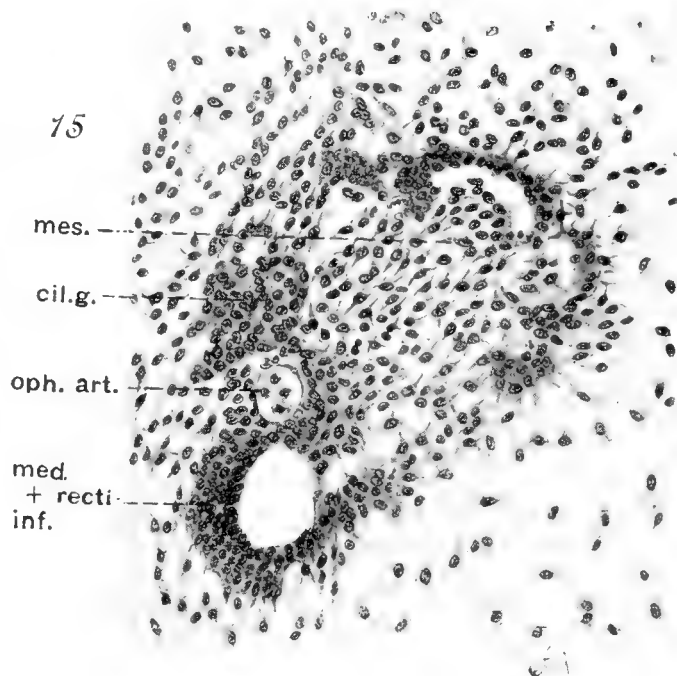
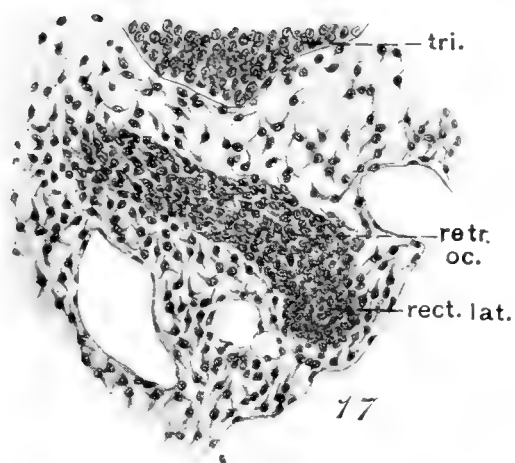


PLATE 5

EXPLANATION OF FIGURES

- 19 Wax plate reconstruction of right side of head of 5-mm. *Chelydra serpentina*. $\times 160$.

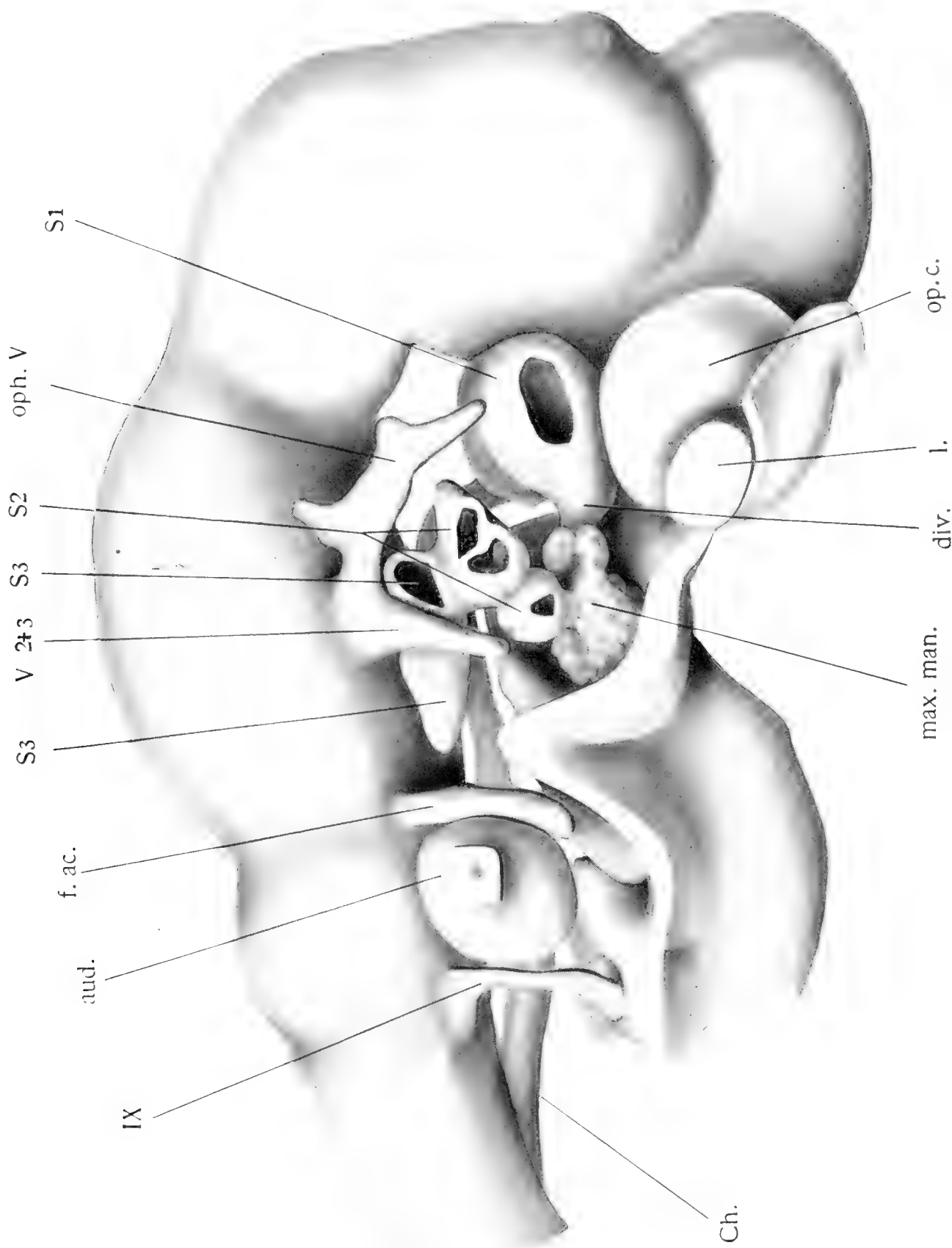


PLATE 6

EXPLANATION OF FIGURES

20 Wax plate reconstruction of right side of head of Chelydra embryo of 9-mm. $\times 80$.

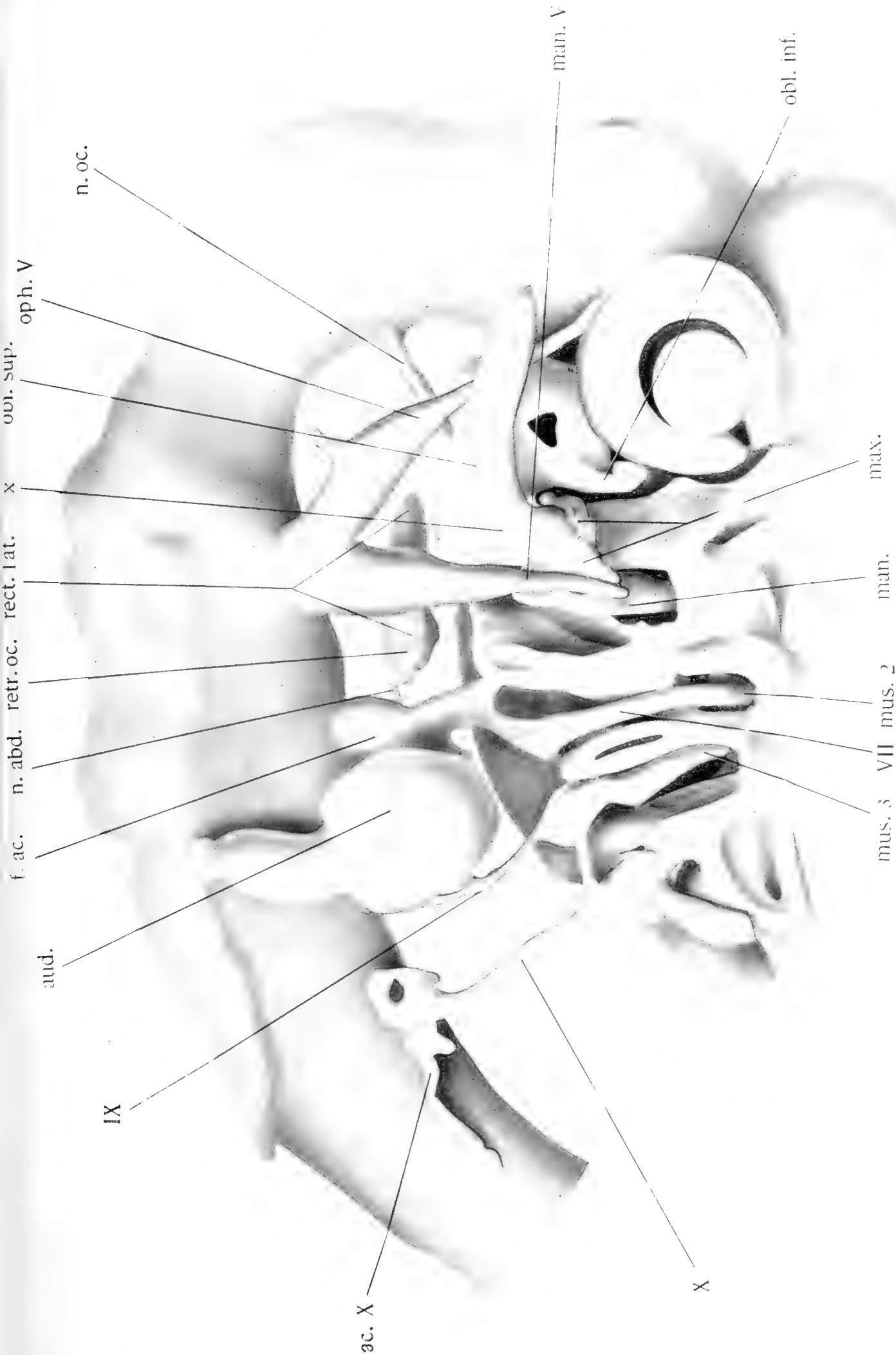
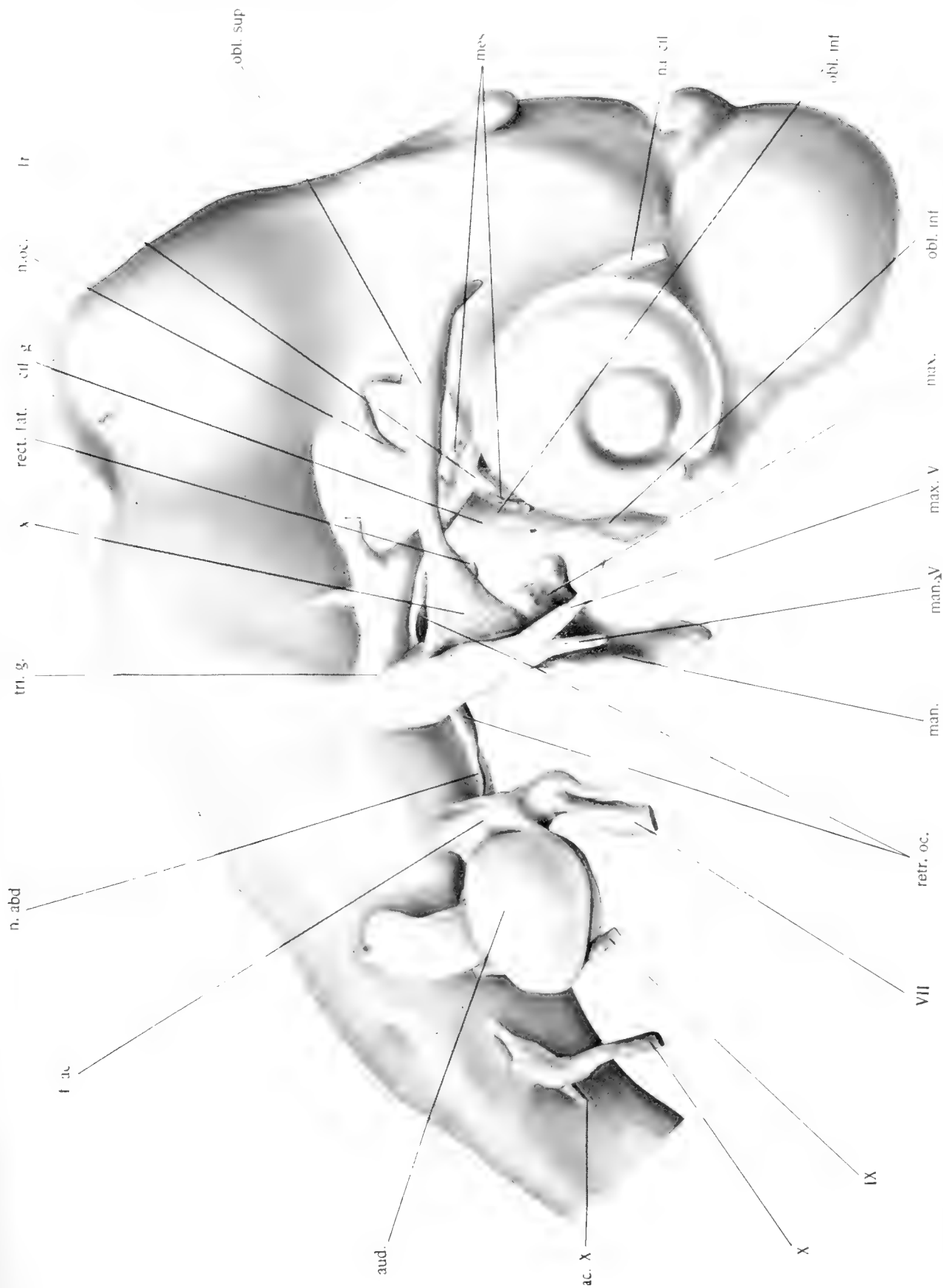


PLATE 7

EXPLANATION OF FIGURES

21 a Wax plate reconstruction of head of 10-mm. embryo Chelydra, showing head somites and developing eye muscles. $\times 50$.

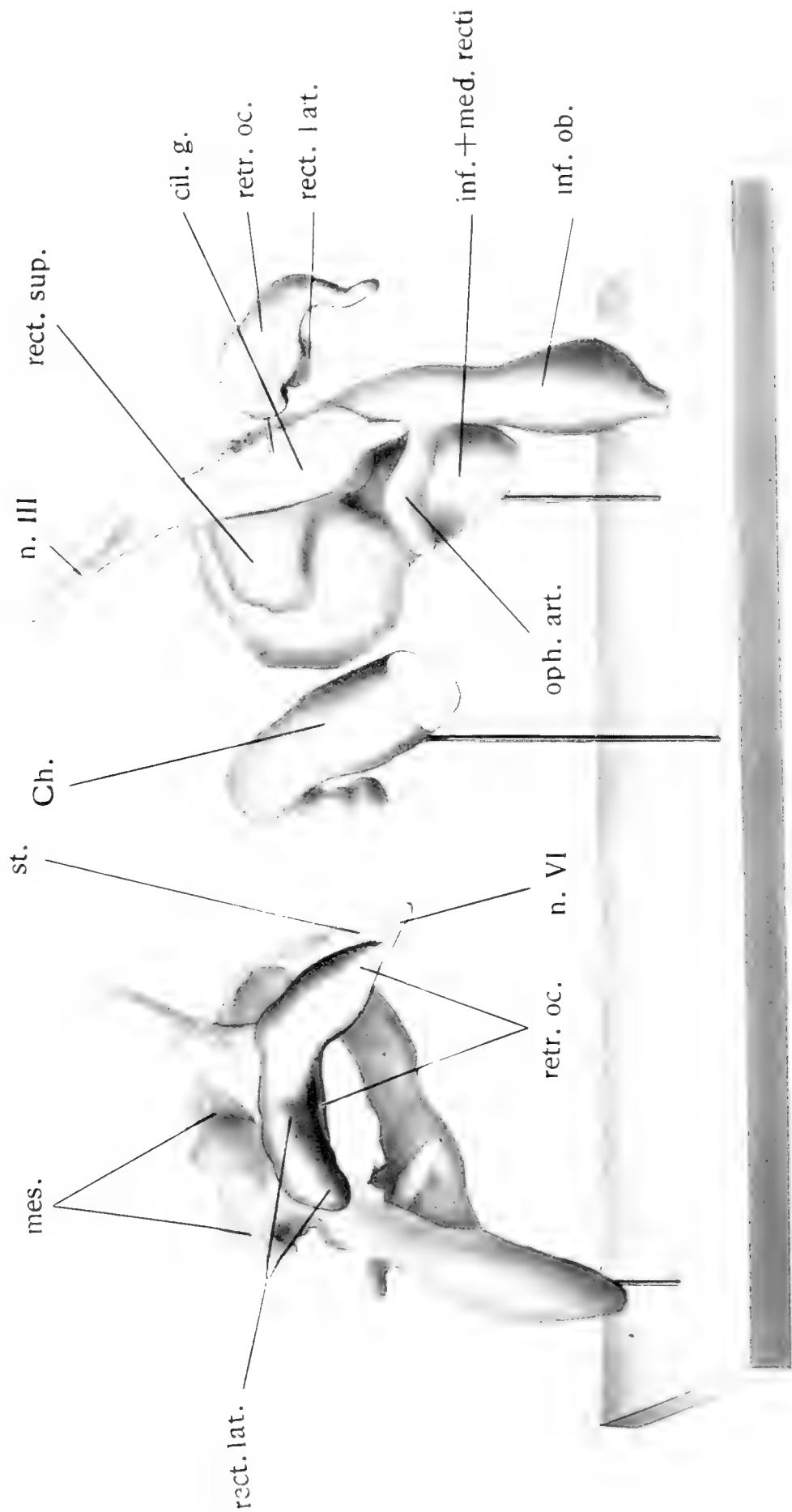


21a

PLATE 8

EXPLANATION OF FIGURES

21 b Wax plate reconstruction of the first and third head somites, with certain adjacent structures, of the 10-mm. Chelydra shown in figure 21 a. Seen from the posterior side. $\times 100$.

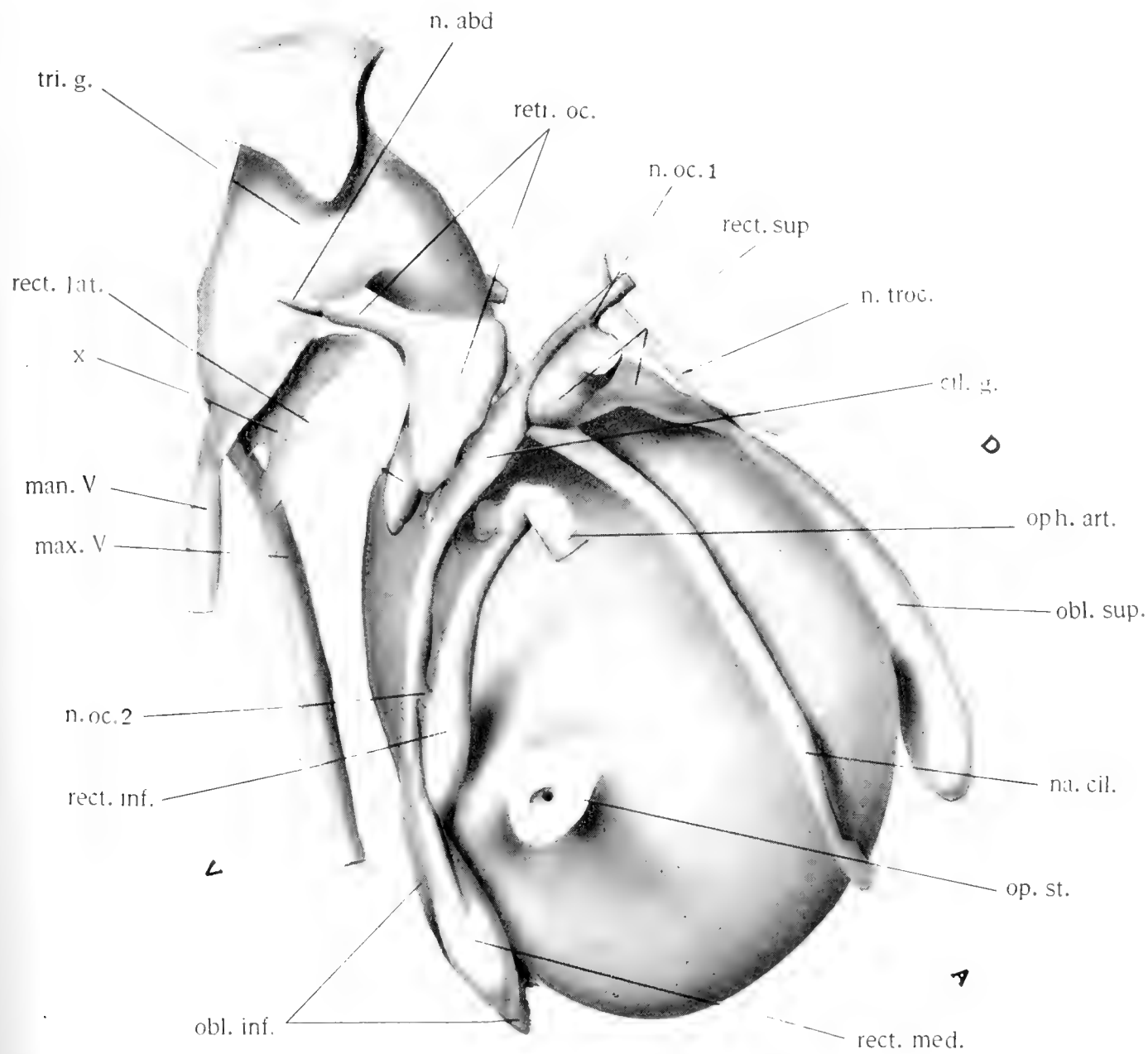


21b

PLATE 9

EXPLANATION OF FIGURES

22 Wax plate reconstruction of the left eye-ball and adjacent structures of an embryo Chelydra of 11-mm. Seen from the median side. $\times 66$. On account of the cephalic flexure, when the embryo is in the upright position and viewed from the side, dorsal and ventral, in figures 22 and 23, are as indicated on the plates, *D.V.*

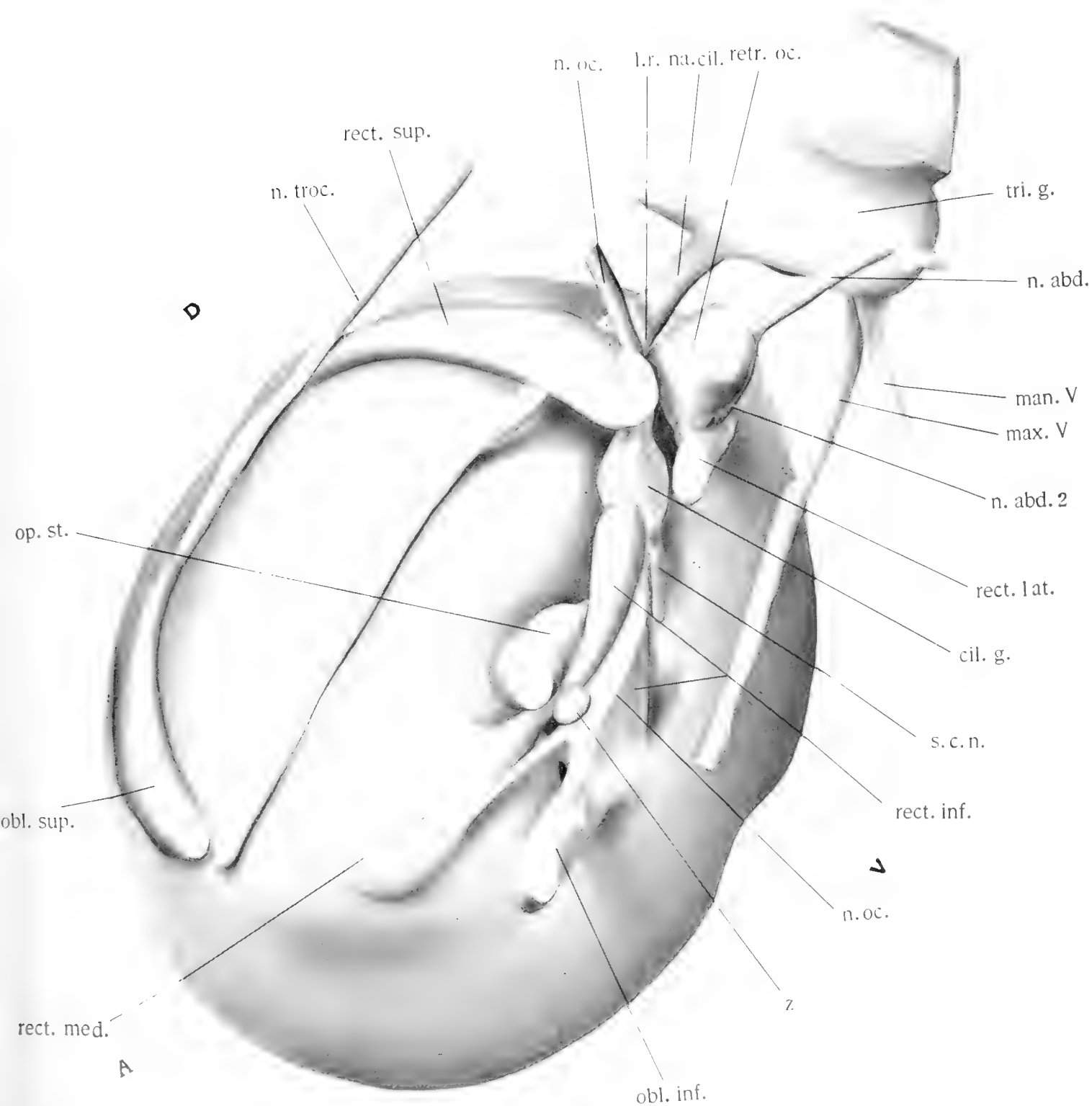


22

PLATE 10

EXPLANATION OF FIGURES

23 Wax plate reconstruction of the right eye-ball and adjacent structures of an embryo *Chrysemys marginata* of 9 mm., Harvard Embryological Collection, series 1085. Seen from the median side. $\times 66$.





THE DEVELOPMENT OF THE MUCOUS MEMBRANE OF THE LARGE INTESTINE AND VERMIFORM PROCESS IN THE HUMAN EMBRYO

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TWENTY-NINE FIGURES

The following paper is the second of a series of studies concerning the mucous membrane of the digestive tract. The first (Johnson '10) dealt with the development of the oesophagus, stomach and small intestine. The present paper is devoted entirely to the large intestine, and includes all its parts except the lower portion of the rectum. It is followed by an account of the effects of distention upon the small and large intestines of various animals. It is proposed to publish later an account of the development of the anal portion of the rectum, work upon which is nearly completed. The study of the mucous membrane of the digestive tract was proposed to me by Dr. F. T. Lewis in 1909, and as the work has progressed, I have received from him many valuable suggestions.

The development of the large intestine is of special interest owing to the variety of pictures its mucosa presents. Relatively simple in the beginning and again in the adult as compared with other portions of the alimentary canal, the mucosa passes through a number of complicated changes before it attains its full development. To begin with, the smooth epithelial tube of the large intestine develops more or less regular longitudinal folds. These folds, as will be subsequently described, are later replaced by villi. Still later the villi themselves disappear. Meanwhile, glands are forming, and when the villi have entirely faded out,

the large intestine reaches its adult condition. The presence of 'transitory' villi has long been known, being first described by Barth in 1868. Since then they have been studied by a number of investigators, and several opinions have arisen in regard to their manner of disappearance. However, most of the former work has been done on lower animals, no one account giving a complete history of the changes that take place in the human embryo.

In the present paper an attempt is made to describe in some detail the mucosa of the large intestine and vermiform process in a number of consecutive embryonic stages, and to present a series of pictures made from wax reconstructions illustrating the descriptions. The following account deals strictly with the human embryo, and is based on a number of carefully selected stages. The embryos used are arbitrarily divided into two groups;—the younger stages, which were sectioned whole; and the older stages, from which the various portions of the digestive tube were removed from the embryo and sectioned separately. The younger stages used were all obtained from the Harvard Embryological Collection. They are as follows:

CROWN-RUMP LENGTH	H.E.C. SERIES NO.	FIXATION
<i>mm.</i>		
7.5	256	Zenker's fluid
10.0	1000	Zenker's fluid
16.0	1322	Picro-sulphuric
22.8	871	Alcohol and formalin
30.0	913	Formalin
37.0	820	Parker's fluid
42.0	838	Zenker's fluid

The older stages were obtained from three different collections. I wish here to express my thanks to Prof. C. S. Minot, Harvard Medical School, Prof. C. M. Jackson, University of Missouri, and Prof. Franz Keibel, Freiburg i/Br., for allowing me the privilege of cutting out what portions of their embryos I desired for my work. The list of older stages used is as follows:

CROWN-RUMP LENGTH	FIXATION	COLLECTION	SERIES NO.
<i>mm.</i>			
50	Zenker's fluid	Keibel	249
55	Alcohol	Minot	
58	Zenker's fluid	Keibel	
65	Zenker's fluid	Keibel	
70	Alcohol	Keibel	
73	Picro-sulphuric	Minot	116
75	Alcohol	Minot	110
88	Formalin	Jackson	137
99	Alcohol	Minot	340
110	Formalin	Jackson	143
120	Zenker's fluid	Minot	342
140	Formalin	Jackson	263
170	Formalin	Jackson	222
187	Formalin	Minot	315
190	Formalin	Jackson	89
200	Formalin	Jackson	
240	Müller's fluid	Minot	186
320	Formalin ¹	Jackson	16
Birth ²	Zenker's fluid	Minot	345
Birth ³	Zenker's fluid	Minot	341
Two weeks child ⁴ ...	Zenker's fluid	Minot	

¹ First injected with a mixture of phenol, alcohol, glycerine, and formalin

² Premature birth at seven (?) months. Lived thirty minutes

³ Normal fetus at birth

⁴ Premature at seven months. Lived two weeks

THE LARGE INTESTINE

Early development

In an embryo of 7.5 mm. the large intestine, like the oesophagus, stomach, and small intestine of the same embryo, is a simple tube of epithelium surrounded by mesenchyma. It is continuous, without demarcation, with the small intestine above, and with the urogenital sinus below. Its cephalic end is indistinctly indicated by a slight swelling, which is regarded by Lewis ('11) as the beginning of the vermiform process. This swelling, which I will designate by the term 'colic ampulla' (ampulla coli), is spindle-shaped and has a diameter (measured from side to side in its widest place) of 0.07 mm. Below the swelling the tube becomes markedly narrower. In its narrowest portion it

measures but 0.04 mm. in diameter. As the urogenital sinus is approached the epithelial tube again increases in size and becomes compressed from side to side. This enlargement passes insensibly over into the epithelium of the cloaca.

The walls of the epithelial tube also vary in thickness. Above, where it is broadest, the walls have a thickness of about 0.028 mm. and are composed of cells which have no distinct cell boundaries. Three rows of oval nuclei are discernible. In the narrower middle portion the walls average about 0.017 mm. in thickness, and show only two rows of nuclei. Where the tube is expanded in the cloacal region, the epithelium is approximately of the same thickness as in the colic ampulla. The lumen in most places is a narrow slit-like cleft, larger in the extremities than in the mid-region of the large intestine, but everywhere present and patent. It communicates with the larger lumen of the cloaca, but cannot be traced through this to the outside because of the presence of the cloacal membrane.

In an embryo of 10 mm. the large intestine presents practically the same relations, but shows a marked increase in size. The colic ampulla, which is now situated well out in the coelom of the umbilical cord, measures about 0.33 mm. in diameter, and has an epithelium 0.045 mm. in thickness. A slight bud-like protuberance of almost the same size as the swelling itself, arises from it, and extends into the mesenchyma. Followed caudally, the epithelial tube of the large intestine quickly diminishes in size, and continues of small size until the region of the rectum is reached. Here it presents another spindle-shaped swelling. This swelling is connected with the cloaca by a short and narrow tube. The upper narrow portion of the epithelial tube measures about 0.07 mm. in diameter, and its wall is 0.028 mm. thick. The swelling is 0.12 mm. in diameter, and has a wall thickness of 0.036 mm. The lumen is continuous as far as the cloacal membrane where it is closed off from the exterior.

At 16 mm. the colic ampulla is similar in form but larger than in the preceding stages, being now 0.17 mm. in diameter. It is directly continued into the vermiform process, which is found

in the umbilical cord, pointing away from the embryo. Large at its base where it joins the colic ampulla, the vermiform process tapers gradually towards its blind end. The before-mentioned narrow portion of the large intestine, now 0.09 mm. in diameter, has increased much in length. The lumen of the lower end of the digestive tube no longer leads into the cloaca, but opens to the outside by an extremely small aperture.

In the further course of its development, the swelling in the rectal region becomes much larger, and longitudinal folds make their appearance. These longitudinal folds increase in numbers, and are markedly constant in position in all the older stages. Just what is the fate of these folds I am unable at the present time to state precisely, but it is not improbable that they give rise to the rectal columns (*columnae rectales* Morgagni) while the spaces between them no doubt develop into the rectal sinuses (*sinus rectales*). A discussion of the further development of this portion of the digestive tract, however, has been omitted from the present paper.

The development of longitudinal folds

In an embryo of 22.8 mm. one sees for the first time a change in the form of the epithelium. In the colic ampulla, which now has a diameter of 0.20 mm., the epithelium shows three low longitudinal ridges on its inner surface. These ridges also extend for short distances into the colon and vermiform process.

It becomes necessary at this point to explain the manner in which the terms 'ridges' and 'folds' have been used throughout the remainder of this article. The term 'ridge' has been employed to designate a thickening of the epithelium which projects into the lumen. It must have no corresponding indentation on its under surface into which mesenchyma would extend. By a 'fold' is meant a projection with an indented basal surface, into which the underlying mesenchyma protrudes. This distinction is desirable, as its usage makes it possible to explain in few words the shape of the basal surface of the epithelium along with that of its free surface.

The epithelium in the embryo under consideration (22.8 mm.) varies in thickness in different regions. In the colic ampulla and vermiform process it is 0.056 mm. thick and shows three to four rows of nuclei. In the remainder of the colon down to the rectal ampulla, it is only 0.034 mm. in thickness and shows but two to three rows of nuclei. At no place are the boundaries between the cells distinct, but the free and basal surfaces are well marked.

In an embryo of 30 mm. the whole of the vermiform process and the valve of the colon lie in the coelom of the umbilical cord. An examination of the interior of the colon in this region shows the beginnings of two to three epithelial ridges. These vary in height, the epithelium being 0.070 mm. thick, measured through the summit of the largest ridge, while in the depressions between them, it is only about 0.028 mm. thick. Three or four rows of nuclei can be made out. The remainder of the colon has an average diameter of 0.15 mm. Throughout its great length the epithelial tube is still cylindrical in shape, having a wall 0.048 mm. in thickness. Three or four rows of nuclei are present.

At 37 mm. the whole of the vermiform process still lies in the umbilical cord. The epithelial ridges are higher than in the former stages. In the base of the vermiform process there are four of these, in the middle only three; in the beginning of the colon, three or four. The highest ridge measures about 0.10 mm. in height and has three or four rows of nuclei. Between the ridges the epithelium is only 0.042 mm. thick and shows but two or three nuclear layers. The remainder of the large intestine gradually decreases in size when followed caudally. The portion which is to become the ascending colon is 0.27 mm. in diameter; the transverse 0.22 mm.; and the descending colon 0.18 mm. The epithelium is about 0.056 mm. thick in all these portions. A few vacuoles, such as have been found in the epithelial walls of the stomach and oesophagus are present in the colon of this embryo.

In an embryo of 42 mm. the ridges of the epithelium are in part replaced by true longitudinal folds. In the vermiform proc-

ess and ascending colon three to four of these are present; in the transverse colon two to three; in the greater part of the descending colon three; while in the remainder of the descending colon five to six more irregular ones. These folds vary in height from 0.014 to 0.028 mm. The epithelium is thicker on their crests than between them. It presents an appearance which is largely in accordance with a condition which Patzelt ('83) has found in the large intestine of the cat embryo. He describes two types of cells. In the corners of the star-shaped lumen the cells are short and broad, and have basal nuclei which stain intensely with haemotoxylin. The cells of the second type are found on the tops of the folds. They are longer, finely granular, and somewhat denser. Their nuclei are long-oval or drop-shaped and stain more intensely than those of the first type. The former groups of cells he states are the first anlagen of the Lieberkühn glands; the latter of the villi. The epithelium of the large intestine of the embryo under consideration (42 mm.) has been described and pictured by Lewis ('11). The two types of cells are found arranged in separate groups, but, however, are not as distinct as those of the cat described by Patzelt.

• In the ascending colon of an embryo of 50 mm., the epithelial tube has a diameter of about 0.23 mm., and shows four distinct longitudinal folds. These are, as shown in figure 12, rounded on their tops, and are of different heights, the largest measuring about 0.06 mm. In the piece of ascending colon sectioned for study, which measures about 0.7 mm. in length, the epithelial tube changes but little in shape, the four distinct longitudinal folds running throughout. The epithelium, which has an average thickness of 0.050 mm., is columnar, and, as seen in sections ten microns thick, is apparently stratified, being composed of two or perhaps three, layers of cells. The nuclei, which are oval in shape, are all placed in a zone midway between the free and basal surfaces of the epithelium, there being a clear zone of protoplasm on either side. A definite cuticular border is everywhere present on the free surface of the epithelium. Two distinct types of cells are not visible. Outside of the epithelium is a zone of loose mesenchyma which is bounded by a thin layer

of myoblasts, the circular layer of the muscularis. This is in turn bounded by a layer of mesenchyma and surrounding the whole, except at its mesenteric attachment, a distinct serous epithelium is seen.

In passing ab-orally from the ascending colon into the transverse colon, one of the four longitudinal folds just described drops out, while a second becomes so much reduced in size that it is scarcely recognizable as a fold (figs. 1 and 13). The two remaining folds are distinct and about 0.06 mm. in height. The epithelial tube has a diameter of about 0.27 mm. The remaining features of this portion of the large intestine are similar to those of the ascending colon.

First appearance of goblet cells

In the iliac colon (50 mm. embryo), the epithelial tube as a whole is flattened from side to side. Its greater diameter is 0.36 mm., its lesser 0.18 mm. A considerable change in the condition of folds is evident. They are shallow, irregular, and more numerous than in the ascending and transverse colons. A model of a small portion of this region is shown in figure 14. The distinction between epithelial ridges and folds is here apparent—only those protuberances, which have indented basal surfaces into which the mesenchyma extends, being considered as true folds. Measured through the ridges the epithelium is in places 0.084 mm. thick, while in the clefts between them, it is only 0.028 mm. thick. The two types of cells described by Lewis in the 42 mm. stage are distinct. A few cells on the ridges have a protoplasm which is clearer than others, and are shaped somewhat like goblet cells. Because these cells in the next few stages take on more and more the appearance of goblet cells until their identity cannot be doubted, I believe them to be goblet cells in a very early stage of differentiation. Voigt ('99) was able to distinguish goblet cells first in the rectum of a human embryo of 70 mm.

The ascending colon of an embryo of 55 mm. has a diameter of 0.45 mm. Ten to twelve longitudinal ridges are found, but

distinct folds are absent. The ridges are irregular in form and of varying size, the largest being about 0.10 mm. in height. The two types of cells are distinct now in this region of the large intestine, some of which are like those which Patzelt has described as drop-shaped. In many places large vacuoles similar to those described above are found in the epithelium of the ridges.

The epithelial tube of the transverse colon is of the same size as the ascending, and shows six well marked projections into the lumen, two of which are folds. In the upper part of the descending colon, two ridges and two folds are present. In the iliac colon the folds drop out and only ridges are found. When

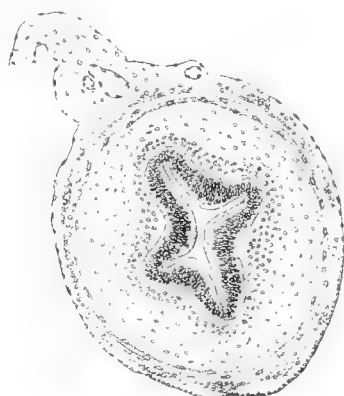


Fig. 1¹ Cross section of the transverse colon of a human embryo of 50 mm. $\times 60$.

followed downward, the descending colon shows more and more ridges and when the sigmoid colon is reached there are as many as ten or twelve. Still more caudally the rectum shows folds which have taken the place of the ridges. In the lower part of the rectum, just above the rectal ampulla, practically all the ridges have been replaced by folds, varying from ten to fourteen in number. The appearance obtained from cross sections, therefore, is somewhat similar to that found in the stomach of the same and slightly older embryos—the clefts in between the ridges corresponding to the gastric pits. The clefts, however, are broader and the cells of the epithelium lining them are more

¹ In this and all remaining text figures certain histological details have been omitted.

columnar in form than those of the gastric pits. Throughout all these portions of the large intestine the cells on the crests of the ridges differ from those between them. In the rectum the epithelium is distinctly one-layered. On the crests of its folds it presents a number of cells with clear protoplasm and basal nuclei. These presumably are developing goblet cells.

In the colon of an embryo of 58 mm. the epithelial tube is found to be quite similar to that of the 55 mm. embryo just described. It is slightly smaller throughout than in the previous stage, which difference may in part be accounted for by the different kinds of preserving fluids used. In the cephalic end

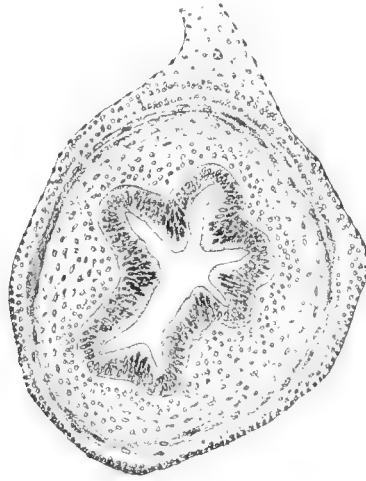


Fig. 2 Cross section of the transverse colon of a human embryo of 58 mm. $\times 60$.

of the ascending colon the epithelial tube has now a diameter of 0.38 mm. and shows numerous ridges and folds as seen in figure 15. More caudally in the ascending colon the epithelium is not so irregular as near the caecum. As seen in sections three folds and one ridge are present. Figure 16 shows a model of this portion of the intestine. It is to be noted that the tops of the longitudinal folds are irregular in form. The diameter of this region of the gut is about 0.36 mm., while the epithelium averages about 0.050 mm. in thickness.

The transverse colon of an embryo of 58 mm. shows six distinct folds, as seen in figure 2. A model of one half of the tube of this region is represented in figure 17. The diameter of the

epithelial tube averages 0.38 mm. The descending colon (fig. 18) has a diameter of 0.34 mm., is more rounded in shape, being quite similar to the more cephalic part of the ascending colon. The crests of the folds and ridges are, however, not so angular.

First appearance of villi

In the rectum the epithelial folds have increased in size and give to the lumen a very irregular form. As shown in figure 19, some of the folds run almost transversely. The presence of transverse folds have been noted in the lower portion of the rectum in a number of older embryos as well. Besides folds, here and there are present conical-shaped projections of the epithelium. These represent the first transitory villi of the large intestine.

In a number of places the folds seem to be fused together at their tops, shutting off small rounded spaces. These spaces I have determined from serial sections to be epithelial cysts. They are found in corresponding portions of the rectum of other embryos, but are confined to this region of the large intestine alone. A portion of one of these cysts is shown in figure 19 at *x*. They are described in detail below.

At this point it seems advisable to make the following summary regarding the development of ridges and folds. In the beginning the epithelial tube is cylindrical in shape. The first changes that take place in its form are found in the rectum, where it shows a number of longitudinal ridges. These ridges are the forerunners of folds, for everywhere they later appear as if pushed in from behind by the underlying mesenchyma. Soon afterward ridges and folds are found in the descending colon, the direction of growth being from below upward. However, before these changes have extended into the transverse colon, similar changes are found to be occurring in the ascending colon near to the colic valve. The direction of growth here is opposite that in the descending colon, that is, ab-orally. The transverse colon is, therefore, the last portion of the large intestine to develop folds. Similarly, in a few of the subsequent stages, the transverse colon shows a slight retardation in the

development of villi and glands as compared with the rectum, descending and ascending colons. However, this retardation is soon overcome by an increased rate in growth, and then conditions found in all parts of the large intestine are quite similar. As a matter of convenience and simplicity, the development of the transverse colon has been described most completely in the remainder of this article, and other portions of the large intestine are described, as far as is possible, from a comparative point of view with respect to it.

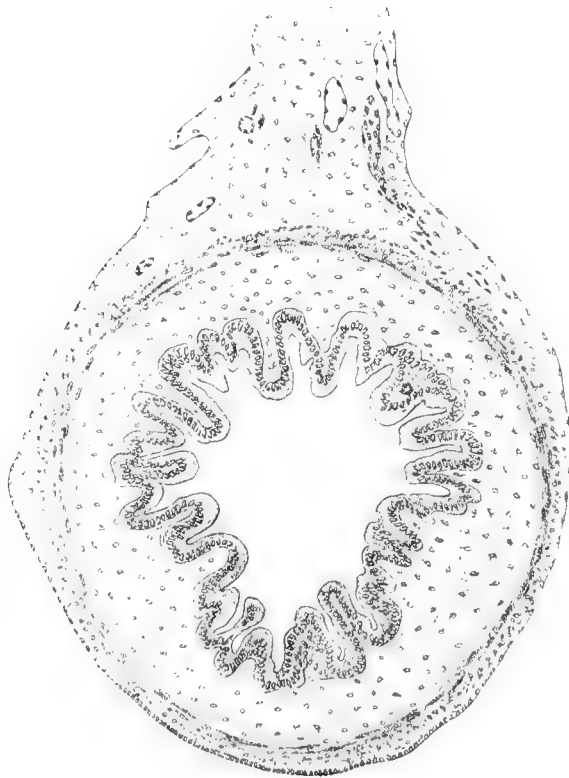


Fig. 3 Cross section of the transverse colon of a human embryo of 65 mm. $\times 60$.

The transverse colon of an embryo of 65 mm. differs considerably from that of the embryo just described. The epithelial tube is circular in section and measures 0.54 mm. in diameter. In transverse section (fig. 3) eighteen to twenty-three projections are seen extending into the lumen. When modelled these projections are seen to be longitudinal folds and villi as shown in figure 20. The villi are everywhere arranged in longitudinal

rows, suggesting that the folds have become broken up into segments. The folds and villi measure from 0.08 to 0.11 mm. in height and are usually between 0.07 and 0.11 mm. in width at their bases. The epithelium on their tops is distinctly simple columnar in form, and is reduced to 0.019 mm. in thickness; between the folds it is 0.031 mm. thick.

Numerous villi are found in the ascending colon. A comparison of these with those villi in the lower part of the ileum shows that the two are quite similar in form and size. At a short distance from the colic valve, the epithelial walls of the ascending colon become pushed in by three large mesenchymal folds, reducing the lumen to a narrow Y-shaped cleft. Here are found folds and villi resembling those shown in figure 20. The descending colon is smaller in diameter than the transverse, being only 0.45 mm. An examination of its inner surface (fig. 21), shows folds and villi, and what apparently are partially formed villi, about eight to nine rows in all. These are longer than those of the transverse colon, 0.17 to 0.22 mm., but of about the same width. In the sigmoid colon is found a condition comparable to that of the descending colon. However, goblet cells are far more numerous.

First appearance of intestinal glands

The epithelial tube of the upper portion of the rectum (embryo of 65 mm.) is flattened from side to side, and measures 1.17 by 0.77 mm. in cross section. A very different appearance is presented from that of the transverse colon. The epithelial wall is bent into a number of folds which are closely packed together. Many of these measure as much as 0.27 and 0.36 mm. in height. The bottoms of the spaces between these projections are developing glands. Where they are cut obliquely or in cross section their basal ends are seen to be tubular in form and provided with small round lumina.

Epithelial cysts are more numerous than in the preceding embryo. They represent glands and intervillous spaces which have become closed over at their tops. They show evidences

of internal pressure by their bulbous appearance and by the flattening of the lining epithelium of the more superficially lying part of the cyst (fig. 11). In many respects these cysts are similar to those found in the vermiform process (compare fig. 11 with figs. 9 and 10) but differ from them by their more superficial position and in that they can rarely be considered to be entirely separated from the surface epithelium. Moreover, they have a different fate from those of the vermiform process. Instead of the epithelium entirely degenerating, the cyst collapsing, and finally being absorbed, the cysts of the rectum open up with the intestinal lumen and become glands again, at least this interpretation seems justifiable, since the cysts gradually disappear without showing such degenerative processes as are easily recognizable in those of the vermiform process.

The condition found in the transverse colon of an embryo of 70 mm. is not much in advance of the same portion of the large intestine at 65 mm. Its epithelial tube has a diameter of 0.54 mm. The lumen is relatively large and the villi project into it 0.09 to 0.13 mm. The cells forming the epithelium are tall columnar, 0.025 in height, and contain at their basal ends, elongated nuclei. The protoplasm, which stains decidedly yellow with orange-G, appears to be mucous in character. Here and there swollen goblet cells are seen. A small portion of the sigmoid colon presents an appearance similar to that described in the rectum at 65 mm. It measures 0.54 mm. by 0.72 mm., and contains folds and villi 0.23 to 0.27 mm. in height. Epithelial glands and cysts are found in large numbers. In the rectum the same conditions are presented, although the epithelial tube is larger and the villi taller (0.25 to 0.32 mm.). The latter seem, however, to be so fused together that they appear in many places as irregular running folds. Goblet cells are everywhere numerous.

In a well preserved transverse colon of an embryo of 73 mm. the epithelial tube measures 0.58 mm. in diameter. The villi and folds, some of which are now 0.22 to 0.23 mm. high, decrease to a marked extent the size of the lumen. In width the villi show a slight increase, being 0.09 to 0.18 mm. through at their

bases. The tops of the villi are in many places so closely approximated that it is quite impossible from cross sections of this stage alone to determine whether an actual fusion has or has not taken place. Because of this condition, which I believe to have been brought about by a strong contraction of the muscularis, an attempt to model these villi accurately proved fruitless. From the conditions found in the large intestine, of other embryos of about the same age, however, it would not seem probable that such a thing as an actual fusion had taken place. The epithelium on the tops of the projections is distinctly one-layered and 0.025 to 0.028 mm. thick, while between them it appears

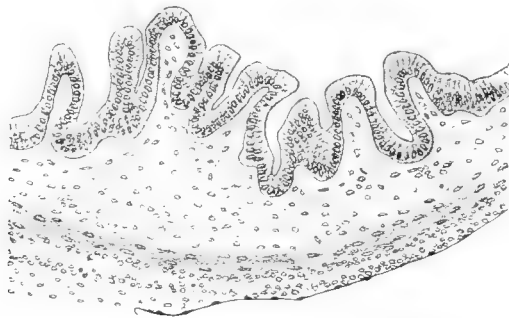


Fig. 4 Cross section of the transverse colon of a human embryo of 88 mm. $\times 60$.

two-layered, and is almost twice as thick, 0.042 to 0.052. Only the portions of the epithelium in between villi are provided with distinct basement membranes.

In the descending colon practically the same conditions are repeated, with the exception that a few more villi are present.

In the transverse colon at 75 mm., even though considerable shrinkage is present, the villi are seen distinctly separated from one another. Other portions of the large intestine from the two last-mentioned embryos were not obtained.

In the transverse colon of an embryo of 88 mm. (fig. 4), are found numerous villi, which are arranged so that they form longitudinal rows. The epithelium on the tops and sides of the villi is similar to that of the preceding stages, being simple columnar in form and containing goblet cells. Between the villi the cells are tall cylindrical and conical in shape, contain oval nuclei

which seem to be closely crowded together, and stain intensively. These groups of cells form small knob-like projections and are the beginnings of the intestinal glands.

In the ascending, descending, and sigmoid colons, as seen from transverse and longitudinal sections, both villi and the beginnings of glands are distinguishable. The villi gradually increase in length as the large intestine is followed caudally; thus in the ascending colon they are about 0.14 to 0.16 mm. in height; in the transverse, 0.18 to 0.20 mm.; in the descending colon, 0.22 to 0.25 mm.; in the sigmoid, 0.27 to 0.32 mm.; while in the rectum, 0.27 to 0.36 mm. In many places their apices are in close contact with each other, appearing as though fused. Likewise, the glands show a more advanced stage of growth as the large intestine is followed downward. In the ascending colon they are scarcely visible; in the rectum they are very distinct. Except for this more advanced stage of development, conditions in the rectum are not so strikingly different from those in the remainder of the colon as at a former period. The epithelial cysts, while not so numerous, have not entirely disappeared. Those few which remain are smaller and are confined to the lower part of the rectum.

In an embryo of 99 mm. the transverse colon shows numerous villi arranged in rows, 20 to 25 in number. As seen in figure 23, few distinct folds are present, but these do not occur in any definite relation to the villi, that is, the rows of villi and the folds are not alternately placed around the wall of the intestine. From this and from what I have seen in other embryos, it seems improbable that the new villi, which are now arising at a very rapid rate, are preceded by folds. More probably they develop after the manner of the villi in the small intestine, as separate growths between the villi already formed.

Numerous gland buds are also present in the specimen as shown in figure 23. Where the glands are cut in cross section, they show small but distinct lumina, surrounded by columnar cells of the mucous variety, many of which are goblet cells. On the tops and sides of the villi the epithelial cells are 0.022 to 0.028 mm. in height while in the glands they are 0.034 to 0.042

mm. Although shrunken from the underlying mesenchyma, the epithelial tube has increased to 1.03 mm. in diameter. The sigmoid colon has a diameter of 0.95 mm. and an epithelium which is similar in variety and of equal thickness to that just described.

From this point on, measurements taken of the glands and villi can only be considered approximately accurate. This is due to the fact that there is no sharp line of demarcation between gland and villus, consequently one is unable to determine just where the gland begins and where the villus leaves off. A similar difficulty was met with in the case of the small intestine. It is, however, possible, with the aid of models for comparison,

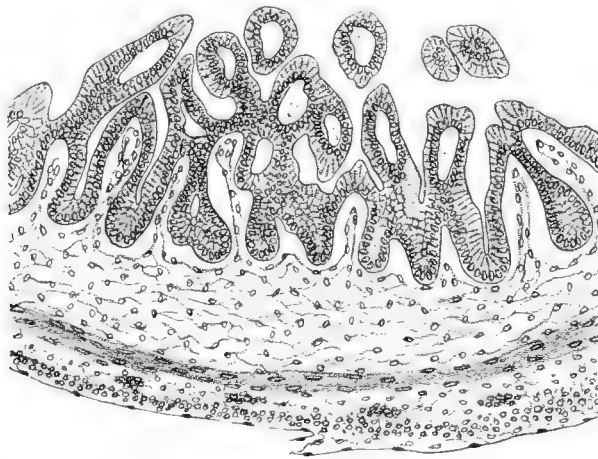


Fig. 5 Cross section of the transverse colon of a human embryo of 110 mm. $\times 60$.

to judge the line of division approximately. In the following account of the growth of glands and villi, the figures given are the average of a number of measurements made from cross or longitudinal sections, or in some cases, from both. A more accurate method, which was employed whenever possible, was the direct measurement of these structures from the models themselves. In the transverse colon of the embryo under description (99 mm.), the glands may be regarded as about 0.07 mm. long and 0.056 mm. broad, while the villi as 0.27 mm. tall and 0.10 mm. broad. In the sigmoid colon the glands average 0.10 mm. and the villi 0.31 mm. in length.

In the transverse colon of an embryo of 110 mm. (fig. 5), the villi are long and narrow, and give to the colon the appearance of a small intestine of a slightly older embryo. Some of them measure as much as 0.36 to 0.45 mm. in length and average about 0.09 mm. in diameter at their bases. They are covered by a low columnar epithelium (cuboidal in places), which is rather poor in goblet cells. The glands are longer than those of the preceding stage, being about 0.13 to 0.16 mm. in length. The cells which line the glands are distinctly columnar, measuring from 0.022 to 0.028 mm. in height. By far the majority of these are goblet cells. Their nuclei are basally placed and closely crowded together, making this region of the gland very deeply stained.

In the ascending colon, a similar picture is obtained. The villi are, however, somewhat shorter (0.27 to 0.36) mm. Noticeable again is the greater distribution of goblet cells in the glands than on the villi, and the difference in the height of the epithelium in the two regions. In the sigmoid colon and the rectum, the epithelial tube is larger and flattened from side to side. The villi and glands are quite similar as regards size, shape and structure to those in the ascending colon. No epithelial cysts were found in the piece of rectum examined, which was a portion taken rather high up.

In a well preserved embryo of 120 mm., the transverse colon has a diameter of about 1.08 mm. in contrast to 1.26 mm. in the preceding embryo. The villi, which are closely packed together, are also shorter (0.18 to 0.27 mm.) than those of the former embryo, but the glands are of about the same length. This difference in size is probably due in part to the different preserving fluids used on the two embryos. The epithelium of both villi and glands is in excellent state of preservation and the goblet cells, which have taken the stain (orange-G) very strongly, stand out in marked contrast to the remaining cells. It is easily seen, therefore, that the goblet cells are more numerous on the sides than on the tops of the villi and most numerous in the glands. In many glands these cells appear to be exclusively present. Although the glands are still only short

knoblike projections (0.11 to 0.14 mm. in length and 0.09 mm. in width), some of them appear to be double. As will be subsequently described, this bifurcating of the glands is a very important factor in their multiplication.

Conditions in the ascending colon, as regards villi and glands, are similar to those in the transverse. In the rectum the epithelial tube is again flattened laterally measuring 2.12 mm. by 0.69 mm. in cross section. The mucous membrane is thicker (0.32 to 0.36 mm.) than that of the transverse colon (0.27 to 0.32 mm.). Its villi are from 0.22 to 0.27 mm. in height and its glands about the same as those found in the transverse colon (0.13 to 0.16 mm.). Goblet cells are again very numerous. A few epithelial cysts were found in the extreme lower part of the rectum, but these were almost insignificant in size as compared to those of the former stages.

Disappearance of villi

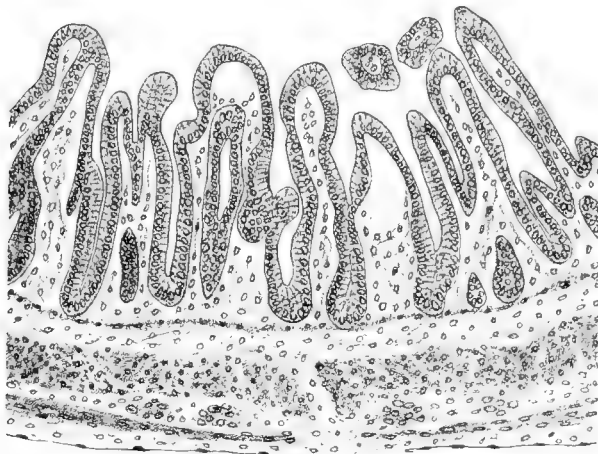
In an embryo of 140 mm. the transverse colon has a diameter of about 1.7 mm. and presents from thirty-six to forty longitudinal rows of villi. It is apparent, therefore, that the villi are still increasing in numbers as the intestine is increasing in size. Since no distinct longitudinal folds can be found in either models or longitudinal sections, these additional villi must develop from the beginning as separate growths in the spaces between those villi already present. The form of the villi is shown in figure 24. Some of the tallest measure only 0.25 mm. in height. Disregarding the embryo of 120 mm., because of the undoubted shrinkage of all its parts, and referring to the 110 mm. stage, it is seen that the villi are shorter in the older embryo. Moreover, some of the villi are so short at 140 mm. that the term villus is scarcely applicable to them, but whether these are newly developed villi or dwindled-down old ones, I am unable to determine. Although the intestinal glands vary in length (0.13 to 0.18 mm.) they are on the whole slightly more advanced than before and many show signs of bifurcating. From the above observations it is evident that, as the glands are in-

creasing in length, the villi are decreasing in height. In the subsequent stages of development, the villi become always lower and lower, so that it is now possible to say that the transitory villi reach their maximum height in embryos between 110 mm. and 140 mm., probably between 110 mm. and 120 mm.

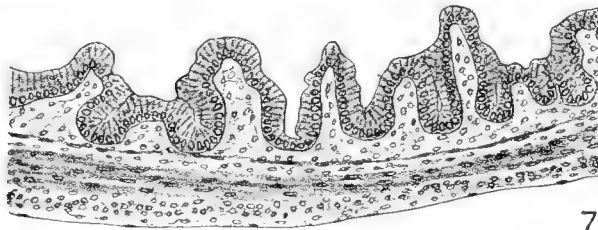
In that portion of the ascending colon adjacent to the colic valve, shorter villi are found (0.14 to 0.18 mm. in height), which are also lower than those in the ileum (0.27 to 0.36 mm.). Higher up in the ascending colon the villi are of about the same size, while the glands are 0.13 to 0.18 mm. in length, and in the beginning of the ascending colon, a few enlarged glands, such as are present in the vermiform process, are found. As regards villi and glands, the descending colon is quite similar to the transverse colon. In the sigmoid colon the villi are longer (0.18 to 0.27 mm.); in the rectum (0.18 to 0.36 mm.). Everywhere, however, the glands remain about the same length as those of the transverse colon. The epithelium is also quite similar throughout the whole colon. It is high columnar in the glands, lower on the sides, and lowest on the apices of the villi. Goblet cells are extremely numerous everywhere, being more abundant in the glands than on the villi.

Effects of distention caused by a storing up of meconium

A marked difference in the thickness of the mucosa is found between the transverse colon of an embryo of 187 mm. and that of the same portion of the intestine of an embryo of 190 mm. (Compare figs. 6 and 7). That of the former is 0.36 mm. in thickness, while that of the latter is only 0.16 to 0.20 mm., the first being about twice the thickness of the second. The question arises, how is this variation to be accounted for? It is to be noted that the portion of intestine of the 190 mm. embryo examined was filled with meconium, thereby extending its walls and increasing the size of its lumen. The total diameter of its epithelial tube is 4.2 mm., in comparison to 2.3 mm. in the 187 mm. stage. These measurements show only that the epithelial tube is extended in the older stage, but they do not show



6



7

Fig. 6 Longitudinal section of the transverse colon of a human embryo of 187 mm. $\times 60$. Shows state of normal contraction (compare villi and glands with those of fig. 7).

Fig. 7 Cross section of the transverse colon of a human embryo of 190 mm. $\times 60$ (compare with fig. 6).

that the circumference of one is greater in one than in the other, because in the younger stage the epithelial wall is thrown into three or four large folds. In order to determine accurately whether the folds alone would compensate for so great a difference in diameter, enlarged camera drawings were made, and the length of the lines at the bases of the glands (the line at which the muscularis is just beginning to appear) was measured. The distended gut (190 mm. stage) measured 15.3 mm., while the contracted one only 11.3 mm., showing still a considerable unaccountable difference. From what I have seen in this and other sections, in the small as well as in the large intestine, I have come to the conclusion that wherever the embryonic intestine is greatly distended with meconium, as is often found to be the case in the older stages, the thickness of the mucosa

becomes greatly reduced. In other words, where a considerable amount of meconium is found in the intestine, the thickness of its mucosa varies indirectly with the amount of distension.

Since the perimeters of the two colons under discussion were unequal in length, it is of interest to compare the number of glands present in them. This was done by counting the number of glands which were practically in contact with the developing muscularis mucosae. This method would not have given comparable results had not the thickness of the sections in both cases been the same (8 microns). In the distended intestine variations from 79 to 96 and an average of 86 were obtained; in the non-distended piece, a variation from 81 to 91 and an average of 85.2, showing that the number of glands is approximately the same. Whether the number of villi around the intestinal wall is the same in both of the intestines, is a harder problem to determine, owing to their greater size and irregularity in height, and to the fact that one is given no distinct basal line, as in the case of the glands, from which to measure. Such a problem could only be determined with any degree of accuracy by making a number of models of comparatively large areas. However, from cross sections alone, it is possible to make out that in the distended intestine the villi are further apart.

In the spreading or stretching out of the mucosa, both the villi and glands become reduced in length and broadened. The following measurements have been made to show this:

	HEIGHT OF TALLEST VILLI	WIDTH OF BASES OF VILLI	LENGTH OF GLANDS	WIDTH OF GLANDS
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Non-distended . .	0.18-0.27	0.07-0.09	0.22	0.05-0.09
Distended	0.00-0.09	0.14-0.18	0.11-0.14	0.09-0.14

It must be noted that the villi had in many places practically disappeared.

The outer layers of the intestine are also reduced in thickness by distension, a fact which is of common observance in the digestive tube of the adult. In the non-distended transverse

colon the submucosa, the muscularis, and the serosa together measure about 0.24 mm., the distended one, 0.13 mm.

From the above given figures, the effects of distention, caused by a storing up of meconium in the large intestine may be enumerated as follows: (1) an increase in the diameter of the epithelial tube; (2) an increase in the actual surface on which the basal ends of the glands lie; (3) a decrease in the thickness of the mucous membrane as a whole; (4) a decrease in the length of the glands and an increase in their width; (5) a more marked decrease in the height of the villi and an increase in their width (6) a spreading apart of the glands and of the villi; and (7) a decrease in thickness in the outer coats of the intestine.

The above results led to the question as to what effect distention would have upon the intestine of an adult. The small intestine of a guinea-pig was experimentally distended with normal salt solution and fixed and hardened in that condition. Although the results of this and other experiments may be found in the following paper, it may be said here, that the results obtained are largely in accordance with those just described in the meconium-filled intestine of the human embryo.

Because of the above described effects of marked distention of the large intestine, it has been considered necessary, for the sake of comparison, to select only non-distended portions of the colon for modelling. In the case of the embryo of 190 mm. the sigmoid colon was chosen, for while this region contains meconium, there is no distention. This is made evident by the presence of longitudinal or oblique folds of the mucous membrane, and by the following measurements as compared with those of the transverse colon of the same embryo: diameter of intestine 3.4 mm.; thickness of mucous membrane 0.34 mm.; thickness of outer intestinal coats, 0.23 mm. Figure 22 shows the epithelium of this region of the intestine in surface view. The villi have dwindled down until they appear merely as irregular knobs which are joined together at their bases in the form of ridges. These ridges are of various lengths but all are of about the same thickness. They run in different directions and anastomose with one another, thus marking the surface of the

epithelium up into an irregular network such as Langer ('87) has described in connection with the developing colic valve. The clefts in between the ridges are deep, and into them open the lumina of the intestinal glands. An examination of the basal surface of another model (not figured), from the same portion of the large intestine, shows numerous tubular glands, many of which are unbranched, but the branched type is not uncommon.

As stated before, the first beginnings of the intestinal glands appear as small knob-like processes which extend into the underlying mesenchyma. At the time they appear no muscularis mucosae is present. In embryos of 99 mm., 110 mm., 140 mm., the muscularis mucosae is still not visible; nevertheless, the intestinal glands all reach a certain depth, so that in a section, a line drawn parallel to the surface of the mucous membrane would practically touch the bottoms of all the glands. It is along this line, or rather, slightly below it, that the muscularis mucosae is becoming visible in an embryo of 187 mm. It is seen as a slight condensation of the mesenchyma forming a circular band of about 0.014 mm. in thickness. In it are observable, though not very distinctly, developing myoblasts. The band is slightly more distinct at 190 mm., and separates the connective tissue of the submucosa, which is condensed and well stained, from that of the tunica propria, which is loosely arranged and only faintly or not at all stained. At 187 and 190 mm. the basal ends of the glands can be seen resting upon this layer of muscle.

Further formation of glands

As the glands are gradually increasing in number as the growth of the embryo proceeds, the question arises, how are new glands formed? Do they develop like new villi, by evaginations of the epithelium between those already formed, or are they developed after the manner which Patzelt has described, by a longitudinal splitting of those already present? In his discussion of this point Patzelt says:

Noch erübrigt es mir, die Art und Weise anzugeben, wie sich die Lieberkühn'schen Drüsen vermehren. Bei der Durchsicht der Präparate, hauptsächlich aus den älteren Stadien, findet man oft im Grunde etwas verbreiterte Drüsen. In der Mitte des verbreiterten Grundes erhebt sich ein Epithelhöckerchen. Oft auch ist dieses Höckerchen nicht mehr blos aus Epithelzellen gebildet, sondern gleicht im Durchschnitte einem mit Epithel überkleideten Zöttchen, welches in das Innere der Drüse hineinragt. Es entspricht dem Durchschnitte eines kleinen Fältchens, welches den Grund der Drüse in zwei Theile spaltet. Dieses Fältchen wächst immer höher und höher. Die Drüse hat endlich das Aussehen, als ob in einem gemeinsamen Vorraum zwei Drüsenschläuche mündeten (Fig. 29 e). Wenn schliesslich die Höhe des Fältchens in gleicher Ebene mit der Innenfläche des Darmes steht, ist der Theilungsprocess vollendet, es sind aus einer Drüse zwei geworden.

He further believes that the upward growing connective tissue papilla continues its upward growth after it reaches the surface level and thus gives rise to a new villus.

In further support of Patzelt's view regarding gland multiplication may be mentioned that if new glands appeared as new outward growths from the epithelium, then one would expect to find always in any fetal intestine glands which extended for varying distances toward the muscularis mucosae. However, such an appearance is never found. The basal ends of the glands, as seen in figures 5, 6, 7, 8, 24 and 25, all extend down to one general level, no intermediate lengths being present. The branched types of glands are always present, some with two, some with three, and some with even four divisions. The amount of bifurcation also varies as Patzelt has figured. As in all problems of growing structures, it is indeed difficult to say precisely what changes are taking place, but I believe it safe to say that in this case that additional glands are developed by a longitudinal splitting of those already present.

In the transverse colon of an embryo of 200 mm. practically the same conditions as those described for the sigmoid colon of the 190 mm. stage are encountered. The mucous membrane, although not thrown up into folds, does not appear stretched out. The villi are of various sizes, the tallest being from 0.14 to 0.18 mm. high. The intestinal glands measure from 0.18 to 0.22 mm. in length. Other measurements taken are as follows:

Diameter of epithelial tube.....	3.5 mm.
Perimeter, measured at bases of glands.....	11.1 mm.
Thickness of mucosa.....	0.40 mm.
Thickness of outer layers.....	0.27 mm.
Number of glands touching muscularis mucosae.....	75.0
Number of glands per running millimeter.....	6.7

In the iliac colon of the same embryo, a slight distention is present as is indicated by the thickness of both the mucosa (0.32 mm.) and the outer layers (0.18 mm.). Although the preservation of the embryo is poor, enough can be made out from it to see that both villi and glands are shorter and broader than in the transverse colon.

In an embryo of 240 mm. the transverse colon shows some distention, but not so much as was seen in this portion of the large intestine at 190 mm. This is made evident by comparing the thicknesses of the mucosa and the outer coats. As seen in a model (not figured), made at double the magnification of the former model, the mucous membrane presents on its lumen surface a number of large irregular anastomosing folds. Between the folds are clefts, which in some places appear to be the glands themselves; in other places they are merely crypts into which the glands open. The glands are, for the greater part, of the simple tubular type, but some show bifurcations into two or three portions. The epithelium, which varies in thickness from 0.020 to 0.034 mm., is made up of the typical simple columnar cells found in the adult intestine, and contains large numbers of goblet cells. The following list of measurements is given for comparison with the other stages:

Total diameter of epithelial tube.....	4.6 mm.
Perimeter, measured at the bases of the glands.....	14.8 mm.
Thickness of mucosa.....	0.36 mm.
Thickness of outer coats.....	0.22 mm.
Number of glands touching muscularis mucosae.....	114.0
Number of glands per running millimeter	7.7

In an embryo of 320 mm. a piece of transverse colon which was greatly distended with meconium was examined. The large folds of the mucosa have all disappeared and the intestinal wall

as a whole is considerably thinned out. Owing to poor preservation, however, it was not possible to determine the presence of villi and folds. Measurements made are as follows:

Total diameter of epithelial tube.....	5.5 x 14.5 mm.
Perimeter, measured as before..	34.7 mm.
Thickness of mucosa.....	0.16 mm.
Thickness of outer coats.....	0.20 mm.
Number of glands	269.0
Number of glands per millimeter.....	7.7

In a fetus of about seven months (premature birth) the ascending colon seems to be almost entirely devoid of villi and the before-described folds. Although not modelled, it is plain to see that the surface epithelium is for the most part, though not entirely, level. At more or less regular intervals, it dips down into the cylindrical intestinal glands. Measurements:

Total diameter of epithelial tube.....	3.7 mm.
Perimeter.....	18.5 mm.
Thickness of mucosa.....	0.27 mm.
Thickness of outer coats.....	0.54 mm.
Number of glands.....	162.0
Number of glands per millimeter.....	8.7

Sections of the sigmoid colon show a similar condition as regards glands. They are of an equal length and similarly distributed.

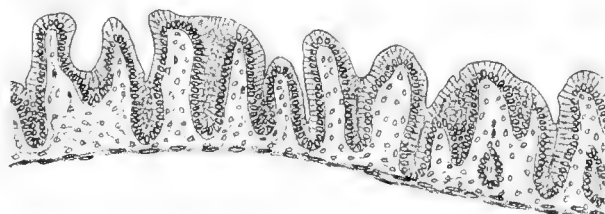


Fig. 8 Cross section of the mucosa of the transverse colon of a human embryo at birth. $\times 60$.

In a fully developed fetus at birth (fig. 8), a condition is reached in which the villi have entirely disappeared. A model of a portion of the epithelium of the transverse colon is shown in figure 25. As seen from the model, only glands are present, the openings of which appear irregular when viewed from the lumen

side. For the most part the mouths of the glands open singly to the surface, but in one region of the model, three can be seen opening together in a slightly depressed region of the surface epithelium. The glands are still of the simple and bifurcated types, the bifurcated ones representing different stages in the process of splitting. The epithelium, which is similar to that in the adult, is 0.017 mm. in thickness at the surface, while in the glands it measures 0.28 mm. Goblet cells are still numerous. Measurements made from cross sections of the transverse colon at birth are as follows:

Diameter of epithelial tube.....	5.5	mm.
Perimeter.....	39.0	mm.
Thickness of mucosa.....	0.25	mm.
Thickness of outer coats.....	0.27-0.72	mm.
Number of glands.....	384.0	
Number of glands per millimeter.....	9.8	

Summary

To the foregoing observations the following summary may be added. As has been pointed out before, the cylindrical tube of epithelium of the early stages develops longitudinal thickenings or ridges. In an embryo of 58 mm. these are becoming partially transformed into low longitudinal folds. In embryos of 65 mm. these folds apparently segment, and in embryos of 88 to 99 mm. true villi are present. We have, therefore, longitudinal folds apparently subdivided to form villi. This view concerning the formation of the villi in the small intestine has been presented by Berry ('00) and confirmed by Forssner ('07). In opposition to this, the view that the villi arise as separate growths of the epithelium, not preceded by folds, has been maintained by Koelliker ('61 and '79), Barth ('68), and Brand ('77). Voigt ('99) believed that the surface epithelium was cut up into a number of elevations by a net work of fissures and furrows, and that these elevations grew into villi. In a study of the development of the whole of the human small intestine the present author ('10) wrote:

In briefly summarizing the development of villi, it may be said that the general tendency throughout the whole of the small intestine is for villi to develop as separate invaginations of the epithelium. Owing however, to the occurrence of transitory structures (vacuoles, diverticula, and folds) their development is manifested differently in different parts of the intestine.

Although in the large intestine formation of villi is preceded by distinct longitudinal folds, it does not seem probable to me, after a study of my models, that there is a mechanical segmentation of the longitudinal folds. It must be remembered that the epithelial tube is ever growing by an increase in the number of its cells, and by the enlargement and duplication of its parts. Because of this active growth one would expect, therefore, villi to form by an active direct process, rather than by an indirect one. It seems more probable to me, that small knob-like elevations are developed along the tops of the folds, and that these knobs form into villi. If such were the case, the picture presented would always be one which would appear like a segmentation of folds. As the villi grew taller and taller, the segmenting fissures would appear to be sinking in deeper and deeper. It is important in this connection to note that the original folds are considerably smaller than the villi, a fact which is in favor of the view just proposed. Regarding the further development of villi, it may be said with certainty, that they arise separately in between those already present.

The earliest glands develop as small knob-like growths of the epithelium into the mesenchyma. As has been pointed out before, the additional glands are probably formed by a longitudinal splitting of those already present.

The villi reach their maximum height in embryos of 110 mm. to 140 mm. in length. From this stage on they gradually become smaller and smaller. A fusion of adjacent villi together at their bases, which gradually extends towards their apices and lengthens the intestinal glands, has been described by Koelliker ('61) and Schultze ('99) as the manner in which they disappear. Brand ('77) describes the connective tissue about the glands as the active factor in their disappearance. He believes that

the connective tissue gradually grows higher and higher, and in so doing, moulds the epithelium of the villi into that of the glands. Schirman ('98) describes the disappearance of the villi of the large intestine of the guinea-pig in an entirely different manner as follows:

Die embryonalen Zotten des Dickdarms bestehen beim Meerschweinchen zu vier Fünfteln ihrer Länge einzig aus Epithelzellen, nur das basale Fünftel der Zotte enthält einen axialen, Blutgefäße führenden Bindegewebestrang. Nur dieses basale Fünftel bleibt erhalten und geht in der Bildung der Lieberkühn'schen Drüsen auf, der grössere Rest wird zurückgebildet, er zerfällt.

It is certain that no such process as Schirman has described takes place in the human large intestine.

The disappearance of villi is indeed difficult to explain in mechanical terms. All that can be said with certainty is that the villi gradually develop, reach a certain maximum height, and then gradually fade out. It is of interest in this connection, however, to note that the effect of distention of the large intestine, caused by a storing up of meconium, brings about a disappearance, or at least a partial disappearance, of the villi. It is hardly probable that mechanical distention of this kind is connected with a lasting disappearance of villi of the large intestine, for they do not disappear permanently from the small intestine, where distentions are also found.

Development of the plicae semilunares coli

The before described longitudinal and oblique folds of the epithelium of the colon are in no way related to the later plicae semilunares. They disappear with the development of villi, and therefore, are only transient structures. The larger plicae semilunares are of later origin, and as they run an oblique course along the wall of the intestine, they have been studied from both cross and longitudinal sections. The observations shown in table 1, have been made to indicate the condition of the inner surface of the large intestine as regards these folds.

TABLE 1
Showing the development of the plicae semilunares

EMBRYO	ASCENDING COLON	TRANSVERSE COLON	DESCENDING COLON	ILIAC COLON
<i>mm.</i>				
65	2 large longitudinal folds	3 large longitudinal folds	None	
65	2 large and 1 small longitudinal fold	2-3 very low folds	None	None
70		1-2 large folds somewhat oblique	1 low broad fold	
88	3 distinct longitudinal folds	2-3 longitudinal folds	Very low, but broad circular folds, 0.09 to 0.13 mm. high	
99		1-2 longitudinal folds		
110	Slight indication of folds			Slight indication of folds
120	None	None	None	None
140		Oblique folds, 0.09 mm. high, 0.90 mm. broad, 0.07-0.14 mm. apart.		
190		None (intestine distended)		Oblique folds, 0.18-0.45 mm. high, 0.27-0.36 mm. broad, 1.0-1.6 mm. apart.
240		Oblique folds, 0.09-0.27 mm. high, 0.36-0.90 mm. broad, 1.2-1.6 mm. apart.		
320		None (intestine distended)		
7 mos. fetus	Oblique folds, 0.36-0.54 mm. high, 0.36 mm. broad, 0.7-1.3 mm. apart.			Oblique folds similar to those in ascending colon.
Birth	Oblique folds, 0.09 mm. high, 0.36-0.45 mm. broad, 1.3-1.4 mm. apart	Oblique folds, 1.3-3.0 mm. high, 0.36-0.54 mm. broad. (seen in cross sections only)	Slight indication of oblique folds	

From table 1 it would appear that the earlier longitudinal folds which develop before the 120 mm. stage are lost. Later oblique folds are formed which, owing to their course, position, and constancy, must be the *plicae semilunares coli*. These folds, however, are not present in portions of the large intestine which are distended, although the opposite view is held by some regarding those of the adult.

THE VERMIFORM PROCESS

Early development

The history of the mucosa of the vermiform process in the human embryo is quite similar to that of the colon. One finds first an epithelial tube with smooth walls and a round lumen. Later as the tube grows in size, folds appear. The folds give place to villi and glands develop. With the development of glands the villi disappear. Unlike the glands of the colon, however, those of the vermiform process oftentimes develop into cysts, which degenerate, and thus cause a decrease in the number of glands. In the following brief description, numerous references are made to the already described conditions of the large intestine.

The early development of the vermiform process has been described in connection with the early stages of the colon. In the last-described stage (42 mm.) it is a simple tube of epithelium of about two to four cell nuclei in thickness, and presents on its inner surface three or four slight ridges. At 55 mm. the epithelial tube is decidedly large near its base (0.60 mm.), but rapidly becomes smaller when followed towards its tip. The epithelium of the basal portion is thrown into ridges which resemble very much those of the ascending colon of the same embryo. A few vacuoles are present in some of the higher ridges. In the base of the vermiform process of an embryo of 58 mm. (diameter only 0.40 mm.) the epithelial ridges are more numerous. They continue distalward for a short distance and gradually fade out.

At 65 mm. the base of the vermiform process measures 0.45 to 0.54 mm. in diameter. Its epithelium is now provided with distinct villi which resemble those found in the ileum and ascending colon of the same stage. Followed distalward the vermiform process becomes somewhat smaller, but even to its end, villi are present. In some places they appear to be forming from longitudinal folds. The epithelium is everywhere simple columnar and has a thickness ranging from 0.028 to 0.042 mm. in thickness, depending whether measured on tops of the folds or between them. The large number of goblet cells present is striking, there being by far a greater number in the vermiform process than in the small or large intestine.

In an embryo of 88 mm. the diameter of the vermiform process at its base is about 0.45 mm. Numerous villi of about the same size as those in the lower part of the ileum at this stage (0.25 to 0.27 mm. in height) are found. The glands have begun to appear and in places where they are cut in cross section they show small round lumina. Toward its tip the villi are distinct, but shorter (0.11 mm. high). Some are in the form of longitudinal folds (fig. 26). Glands are in most places very small, but their beginnings are everywhere visible.

The epithelial tube of the base of the vermiform process in an embryo of 110 mm. has a diameter of about 0.70 mm. Both glands and villi are distinct, but the former are as yet very rudimentary. In most places they are, as well as can be determined from cross and longitudinal sections, 0.09 to 0.11 mm. in length. The villi are shorter than those found in the base of the vermiform process of the preceding stage, being only 0.14 to 0.16 mm. in height. Goblet cells are again very numerous. The distal end has a diameter of 0.77 mm., being slightly greater than its proximal portion. The villi are again shorter (0.11 to 0.13 mm.) than those found above, but somewhat longer than those found in the same portion of the vermiform process of the preceding embryo. The glands are approximately the same length as before. The extreme tip is rounded in shape, and is provided with villi and glands of the same type as those on the side walls.

Enlarged glands and gland cysts

In an embryo of 140 mm. the epithelial tube of the caecum is 1.4 mm. in diameter, and its villi 0.18 mm. to 0.27 mm. high. For the most part the glands are 0.09 to 0.13 mm. in length and 0.054 to 0.072 mm. in width. Some of the glands, however, are swollen at their lower ends, being from 0.09 mm. to 0.13 mm. in diameter. They are all supplied with lumina, which in the enlarged glands are occluded at the necks of the glands. More distally, the base of the vermiform process has a diameter of only 0.9 mm. The villi are similar to those of the caecum, and the bulbous glands are more numerous. Still more distally, near the tip of the vermiform process, the villi are shorter (0.14 to 0.16 mm.), and the enlarged glands are found in still greater numbers. Figure 27, from a model of the epithelium of the tip shows some of these glands. Many have enlarged ends connected with the surface epithelium by constricted necks. The gland marked in the figure is the only gland which could be found which is entirely cut off from the surface epithelium. From serial sections, it is possible to make out that although the bulb-like expansions are provided with decidedly large lumina, these are totally cut off from the lumen of the vermiform process. Moreover, the cells of the necks of the glands appear to be undergoing degenerative changes. In many places only a slight strand of tissue, largely composed of epithelial cell nuclei, could be found connecting the bulb with the surface epithelium.

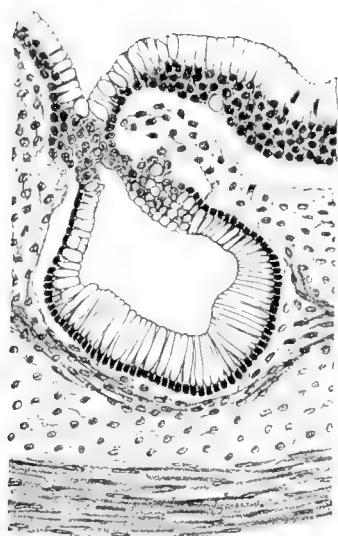
In the vermiform process of an embryo of 170 mm. the enlarged glands are very numerous. Owing, however, to poor preservation it is impossible to state whether they are connected with the surface or not. In the connective tissue of the submucosa can now be seen distinct nodules of lymphoid tissue.

Sections through the base of the vermiform process of an embryo of 190 mm. shows ill-marked villi, 0.07 to 0.11 mm. in height and of variable width. The glands, now 0.11 mm. to 0.16 mm. long, are more regular and only a few tend to be bulbous. The condition found is quite similar to that of the sigmoid colon of the same embryo with the difference that there are

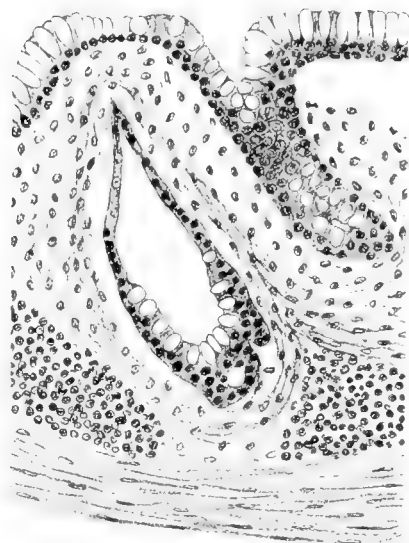
fewer glands present. For the first time, the muscularis mucosae becomes visible, and the glands in places pierce it. In the tip of the vermiform process the epithelial tube is expanded to a diameter of 1.7 mm., in comparison to 0.6 mm. at its base. The villi have practically disappeared, only very slight elevations being left in places. The glands are similar in form, but farther apart than those of the base. In places the muscularis mucosae can be seen, but it is not well differentiated as yet.

In an embryo of 200 mm. the epithelial tube of the base of the vermiform process (fig. 28), although its diameter is about the same (1.6 mm.), is quite different from that of the preceding stage. The villi have almost everywhere entirely disappeared. The glands are of various size, some of the swollen ones being 0.13 to 0.14 mm. broad. Most of these extend down only to the muscularis mucosae, others have pushed into it and cause it to bulge outward, while still others have pierced it. In some places the swollen ends of glands appear as cysts, entirely cut off from the surface epithelium (figs. 10 and 28). Some cysts appear to be entirely surrounded by a thin stratum of smooth muscle derived from the muscularis mucosae. The epithelium lining the cysts shows various stages of degeneration. In some it appears almost similar to the surface epithelium. In others only that part of the epithelium lining the base of the cyst is similar to that of the surface, while the upper portion, that is, that portion of the cyst which had formerly been the neck of the gland, has an epithelium which is much thinner and composed largely of broken down nuclei and fragments of cell protoplasm. In other cysts, degeneration has involved all the cells of the epithelium, but those in the upper end again show a more advanced stage of deterioration. Still other cysts are of smaller size and have their epithelia reduced to a thin line.

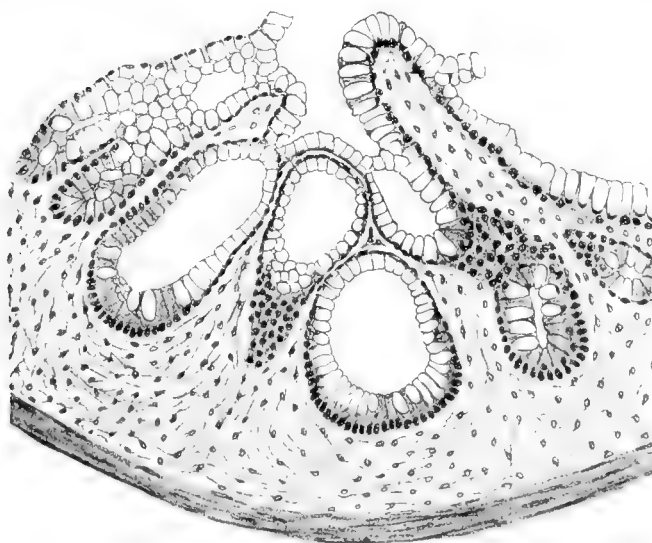
Stöhr ('98) and Nagy ('11) found epithelial cysts of the kind described in the vermiform process of human embryos. According to Stöhr the epithelium of the cut off glands becomes poorer and poorer in goblet cells and begins to degenerate. The cyst itself becomes filled with degenerative products, and as the epithelium gradually goes to pieces the walls collapse. He



9



10



11

Figs. 9 and 10 Cross sections of the mucosa of the vermiform process (including the circular layer of the muscularis). Human embryo of 200 mm. $\times 120$. In figure 19 is shown a gland cyst not entirely cut off from the surface epithelium. In figure 10 is shown a cyst detached from the surface epithelium and undergoing degenerative changes.

Fig. 11 Mucosa of the rectum (including the circular layer of the muscularis) in cross section. Human embryo of 65 mm. $\times 120$. Shows several epithelial gland cysts.

concludes: "Als letzten Rest der Drüse findet man dann minimale, 0.05–0.15 mm. messende Gruppen kleiner Epithelzellen, die von einer dicken bindegewebigen Kapsel (Fig. 19a) umgeben sind."

Numerous and distinct lymphoid nodules are present in the vermiform processes of all the embryos from 170 mm. up, but never, so far as I have observed, are the cysts located in them. I agree with Stöhr when he says: "Der Verfolg der Serie zeigt immer, dass die reducirten Drüsen nur am Rande des Knötchens liegen, zuweilen sogar in dessen Peripherie hineingepresst sind. Das Centrum der Knötchen enthält keine reducirten Drüsen."

Moreover, such a degeneration of glands is not found in other portions of the large intestine where lymphoid nodules are plentiful. It is not probable, therefore, that the development of lymphoid tissue has anything to do with the formation and degeneration of these cysts.

There are no marked changes in the form of the mucosa of the vermiform process in the subsequent stages of development. At 240 mm. villi, bulbous glands, and cysts have entirely disappeared. The glands, as seen in figure 29, are small and rather widely separated. The muscularis mucosae is distinct and occasionally glands can be seen which have pierced it.

In specimens at seven months, birth, and a two weeks old child, practically the same conditions are found. Lymphoid tissue, both in the diffuse and nodular form, is abundant. The statement made by Berry and Lack ('06) that "In the vermiform appendix of the full term fetus there is practically no lymphoid tissue, or at least so little as to constitute a negligible quantity, whilst lymphoid follicles are absent," cannot be confirmed.

CONCLUSIONS

1. The large intestine, like the oesophagus, stomach, and small intestine, is at first a simple tube of epithelium, and is the last portion of the digestive tube to show distinctive changes in the form of its mucosa. The first change which occurs is the formation of longitudinal folds and ridges.

2. The ridges and folds first appear in the rectum and then extend upward (orally). A second point of growth is found in the ascending colon at the colic valve. The direction of growth here is in the opposite direction (aborally). The transverse colon is the last portion of the large intestine to develop ridges and folds.

3. A few vacuoles similar to those found in the oesophagus, stomach, and small intestine, are found in the epithelial wall of the large intestine in its early stages.

4. The longitudinal folds are replaced by villi in a manner which suggests a segmenting of folds. However, it is not improbable that the villi of the large intestine arise after the manner of those of the small intestine, as separate growths along the tops of the folds.

5. Additional villi arise as separate growths between those villi already formed, not being preceded by folds.

6. The first glands appear as knob-like protuberances of the epithelium into the underlying mesenchyma. Additional glands develop by a splitting, from below upwards, of those already formed.

7. Villi reach their maximum height in embryos between 110 mm. and 140 mm. in length. From this time on they gradually become smaller in size. Remnants of them persist as folds which form an irregular network in between the gland openings. They are entirely gone at birth.

8. In the vermiform process villi and glands develop, and the villi disappear at the same time and in the same manner as those of the ascending colon.

9. Enlarged and cystic glands are found in the vermiform process and caecum. Many of them become detached from the

surface epithelium and are then entirely surrounded by connective tissue. They later disappear by degeneration and absorption. Lymphoid nodules are found in abundance in the vermiform process at birth, but the cysts never lie in the centers of them.

10. Epithelial cysts are also found in the mucosa of the rectum. They are in many ways similar to those found in the vermiform process, but differ from them in that they are not so deeply seated in the tunica propria, are seldom entirely detached from the surface epithelium, and disappear not by degeneration and absorption, but by breaking through into the intestinal lumen.

11. Distention of the large intestine, due to the accumulation of meconium, brings about marked changes in the form of the mucosa. It reduces its thickness, broadens and shortens both villi and glands, and causes them to become spread apart.

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PLATE 1

EXPLANATION OF FIGURES

- 12 Wax reconstruction of the epithelium of the ascending colon. Human embryo of 50 mm. $\times 89$.
- 13 Wax reconstruction of the epithelium of the transverse colon. Human embryo of 50 mm. $\times 89$.
- 14 Wax reconstruction of the epithelium of the descending colon. Human embryo of 50 mm. $\times 89$.
- 15 Wax reconstruction of the epithelium of the ascending colon (portion near the colic valve). Human embryo of 58 mm. $\times 89$.
- 16 Wax reconstruction of same. More caudad portion. $\times 89$.
- 17 Wax reconstruction of the epithelium of the transverse colon. Human embryo of 58 mm. $\times 89$.
- 18 Wax reconstruction of the epithelium of the descending colon. Human embryo of 58 mm. $\times 89$.



Fig. 12



Fig. 13

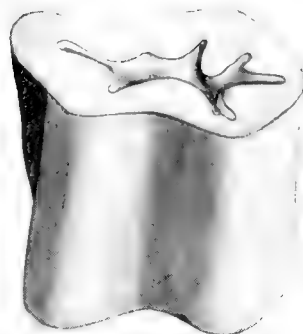


Fig. 14

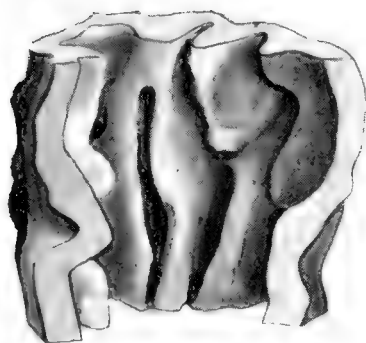


Fig. 15

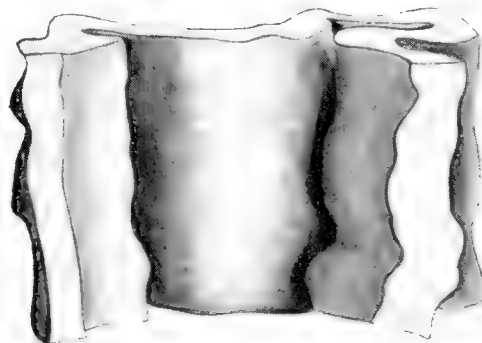


Fig. 16

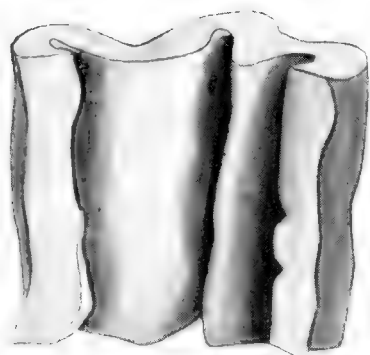


Fig. 17



Fig. 18

PLATE 2

EXPLANATION OF FIGURES

19 Wax reconstruction of the epithelium of the upper part of the rectum. Human embryo of 58 mm. $\times 89$; *x*, epithelial gland cyst.

20 Wax reconstruction of the epithelium of the transverse colon. Human embryo of 65 mm. $\times 89$.

21 Wax reconstruction of the epithelium of the descending colon. Human embryo of 65 mm. $\times 89$.

22 Wax reconstruction of the epithelium of the sigmoid colon. Human embryo of 190 mm. $\times 89$.



Fig. 19

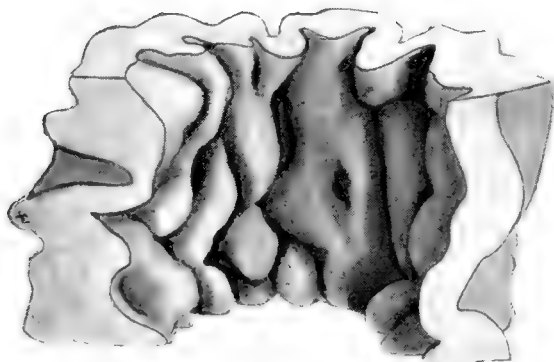


Fig. 20

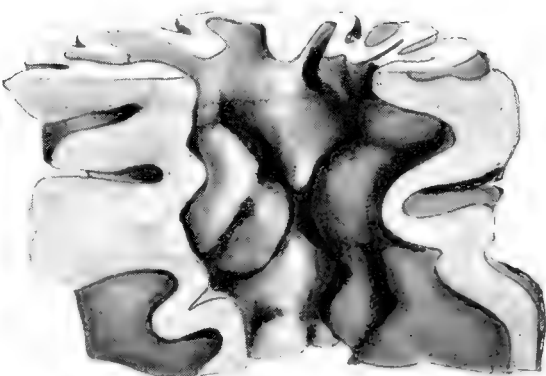


Fig. 21



Fig. 22

PLATE 3

EXPLANATION OF FIGURES

- 23 Wax reconstruction of the epithelium of the transverse colon. Human embryo of 99 mm. $\times 89$.
- 24 Wax reconstruction of the epithelium of the transverse colon. Human embryo of 140 mm. $\times 89$.
- 25 Wax reconstruction of the epithelium of the transverse colon. Human embryo at birth. $\times 178$.



Fig. 23



Fig. 24

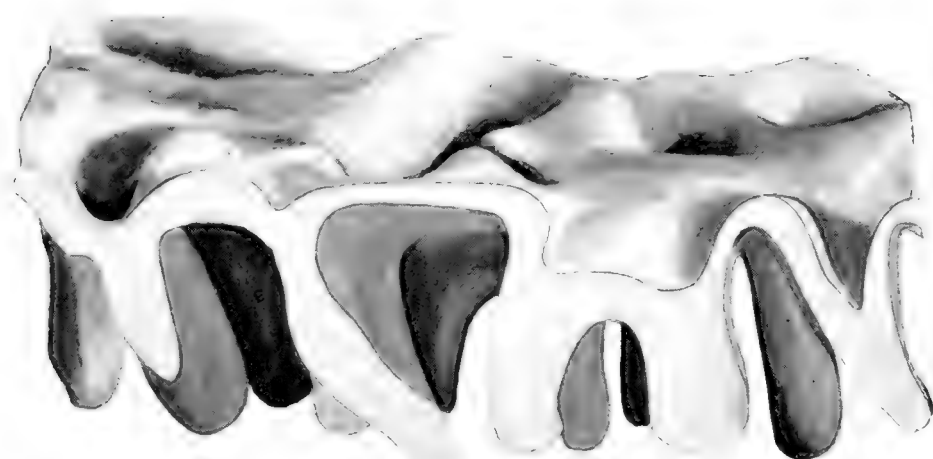


Fig. 25

PLATE 4

EXPLANATION OF FIGURES

26 Wax reconstruction of the epithelium of the tip of the vermiform process. Human embryo of 88 mm. $\times 89$.

27 Wax reconstruction of the epithelium of the tip of the vermiform process. Human embryo of 140 mm. $\times 89$; *x*, epithelial gland cyst, entirely cut off from surface epithelium.

28 Wax reconstruction of the epithelium of the base of the vermiform process. Human embryo of 200 mm. $\times 89$; *x*, epithelial gland cyst, entirely cut off.

29 Wax reconstruction of the epithelium of the tip of the vermiform process. Human embryo of 240 mm. $\times 89$.



Fig. 26



Fig. 27

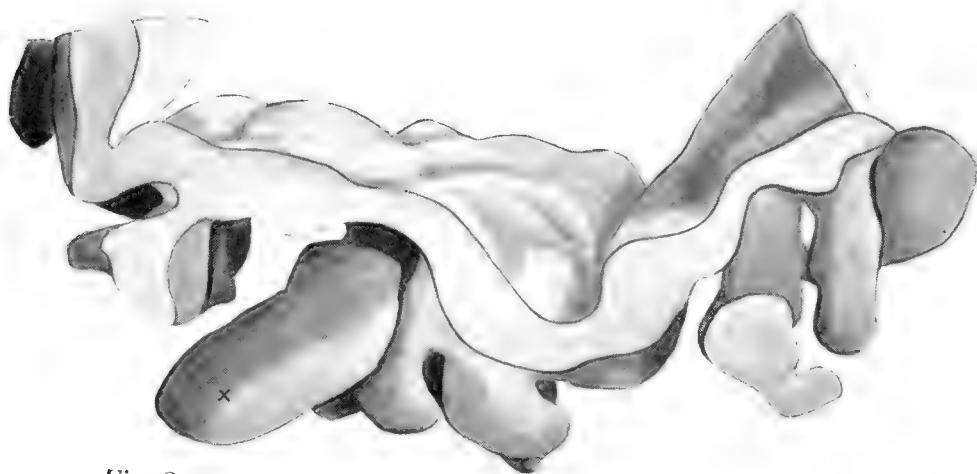


Fig. 28



Fig. 29

THE EFFECTS OF DISTENTION OF THE INTESTINE UPON THE SHAPE OF VILLI AND GLANDS

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ELEVEN FIGURES

While studying the development of the large intestine in the human embryo, I observed a striking difference between the forms of the mucosa of two transverse colons, one of which was filled with meconium, whereas the other was empty. Briefly stated, the distended piece of intestine in comparison with the non-distended, possessed a thinner mucosa, thinner outer coats, shorter and broader villi, and shorter and broader glands. The differences were so marked that it seemed probable from the beginning that they must have been caused by the distention of the intestine. The question arose, thereupon, since a distention of the embryonic intestine may bring about such results, cannot similar changes be produced in the intestine of the adult?

Accordingly, the experiment of distending a portion of the small intestine under pressure and hardening in that condition was attempted. The guinea-pig, being the animal most easily obtained, was fortunately used for the purpose. A portion of the small intestine, 5 to 7 cm. in length, was tied off, and distended through a small canula with normal salt solution. The pressure obtained from a column of water 150 cm. high was used. The distended piece of intestine was then placed under pressure in Zenker's fluid for about one hour, then in alcohol under pressure for several hours. Being hardened in a distended state, the pressure was then removed and the piece of tissue imbedded in paraffin and sectioned. For comparison

an empty piece of intestine, taken immediately adjacent to the distended piece, was treated with the same fixing fluids. Microscopical examination showed most interesting results, as can be seen by comparing figures 1 and 2. The villi have been greatly shortened and the glands stretched out and obliterated. These results led to a number of experiments which are described below.

A survey of the literature shows that the idea that villi are capable of changing their form under different conditions is an old one. These changes, however, have been usually considered to be produced by the intrinsic muscle fibers of the villi. That villi undergo shortening was noted by Lacauchie ('43), who recognized the muscular nature of the walls of the lacteals, by Gruby and Delafond ('43), and by Brücke ('50). Kölliker ('51) showed that the muscle fibers of the villi run down between the intestinal glands and that they are related to the fibers in the muscularis mucosae. By a contraction of these fibers, it was then and is now commonly believed that a shortening and broadening of the villi may be produced.

We owe to Heitzmann ('68), however, the discovery that the shapes of villi vary with the distention of the intestine, a fact which apparently has been lost sight of in the current textbooks and publications. Heitzmann found that a piece of intestine of a freshly killed guinea-pig possessed alternating contracted and distended portions, and that if the piece be thrown into a chromic acid mixture the contracted portions remain permanently contracted, and the distended portions distended. His examination of the villi of the contracted portion showed them to be long and cylindrical; of the distended portion, flat and conical. Moreover, he observed that artificial distention, produced by filling a tied-off piece of intestine with chromic acid mixture beyond the limits of ordinary normal expansion, or extreme distention caused by the formation of gas in the intestine, almost entirely obliterated the villi. This he found to be true not only for the villi of the guinea-pig, but to a less extent for those of the rabbit and cat. He concluded, therefore, that there is no fixed form for the villi, but that their shapes

are dependent upon the contraction of the intestinal tube. He believed that during the movements of normal peristalsis there is a continual changing in the form of the villi. He also noted that the intrinsic muscle fibers of the villi act as the antagonists of those of the muscularis of the intestine wall.

Later, in his "Microscopical morphology of the human body" ('83), Heitzmann expressed the same idea:

The villi are reduplications of the mucosa, of a conical or cylindrical shape, very long and narrow in portions where the muscle of the intestine is contracted; broad and short, on the contrary, where the muscle of the intestine is extended. In the highest degrees of distention (by gaseous material) the inner surface of the mucosa is smooth, and no villi are perceptible.

Heitzmann made no mention of effects of distention upon the shapes of glands.

Verson ('71) in his description of the villi of the small intestine, says:

Sie sind bald cylindrisch, bald kegelförmig, bald keulenförmig oder artig ausgebreitet, was zum Theil vom Contractionzustand der Muskelhäute und ihrer eigenen Muskulatur abhängt und weshalb auch ihre Länge sehr wechselnd ist.

In a study of intestinal contraction Mall ('96) found that the injection of oil into tied-off pieces of intestine of the dog brought about a shortening of both villi and glands and that up to a certain limit, the shortening varied directly with the amount of distention.

Harvey ('08) studied the large intestine of the dog and man and found that the length and breadth of the intestinal glands varied with distention and contraction of the intestinal tube. From a limited number of observations he concluded that the glands of the transverse colon are subject normally to greater changes in length and breadth, and those of the ascending colon to smaller changes, than the glands of other parts of the large intestine. He made no mention of the effects of distention upon the villi and glands of the small intestine.

Bujard ('09) made an extensive study of the villi in a number of vertebrates and as he has given a résumé of the literature on this subject, another need not be presented here. With regard to the villi of mammals Bujard has reached the following conclusions: In herbivorous mammals, where a large residuum of food material is found in the intestine, the intestine is long and the villi small and few. In insectivores, frugivores, and omnivores, where there is only a moderate amount of food material present, the length of the intestine is medium and the villi large and numerous; while in the carnivores, where a minimum residuum of food is left, the intestine is shorter and the villi are narrow, long and pointed, and very numerous. However, Bujard makes no mention of the fact that it is the amount of residuum which determines the amount of distention of the intestine, and that it is this in turn which determines the shape of the villi. He believes, rather, that it is the nature of the food material itself which is the active factor in producing different shapes. To substantiate this view he has performed experiments upon the intestines of white rats, in which he found that after the continued feeding (140 to 380 days) of milk and meat diets, the villi become long and narrow, while on vegetarian diets, the villi become broader and shorter. Unfortunately, in his descriptions of villi, he does not state whether the pieces of intestine he examined were in a contracted, partially contracted, or in a distended condition.

As will be seen from the following experiments, the variations in the form of villi are so great under normal conditions of distention and contraction, that it is necessary, in order to obtain a comparable series from a number of animals, to select pieces of intestine contracted or distended to like extents, and it seems desirable that all experiments of alimentation of the nature of those performed by Bujard, should be preceded by a study of the effects of contraction and distention upon the shapes of the villi and glands.

THE INTESTINE OF THE GUINEA-PIG

Normal contraction

An adult guinea-pig weighing 525 grams was starved for four days in order that the digestive tube might be emptied. Although the stomach and large intestine still contained a moderate amount of food material, the small intestine was almost empty and contracted. Small pieces of the intestine (3 to 5 cm. in length), taken at measured intervals below the pyloric and colic valves, were removed and placed in Zenker's fluid. As shown in table 1 the thickness of the mucosa and the length of the villi and glands diminishes as the colic valve is neared.

TABLE 1

Measurements made from normally contracted pieces of small intestine of the guinea-pig. Total length of small intestine 125 cm.

PIECE OF INTESTINE	MEAN DIAMETER	THICKNESS ¹ OF MUCOSA ²	TALLEST VILLI	DEEPEST GLANDS
	<i>mm</i>	<i>mm</i>	<i>mm</i>	<i>mm</i>
Upper duodenum.....	3.6	1.03	0.72	0.32
10 cm. below pyloric orifice..	2.2	0.95	0.68	0.22
40 cm. below pyloric orifice..	1.5	0.76	0.58	0.22
70 cm. below pyloric orifice..	1.3	0.54	0.36	0.18
90 cm. below pyloric orifice..	1.8	0.68	0.54	0.18
Lowest part of ileum	2.4	0.41	0.31	0.14

¹ Greatest

² Not including muscularis mucosae

The form of the villi as seen in cross section of the intestine is shown in figure 5. They appear as tall and slender projections, but these elevations are in fact zig-zag folds which run transversely around the wall of the intestine. Their true form may be readily seen in whole mounts and has been correctly described by Bujard. Owing to their zig-zag form, the villi present similar appearances when seen in longitudinal or transverse sections of the intestine. In the lower part of the intestine, according to Bujard, the villi tend to be more pointed and conical.

Certain portions of the large intestine examined showed considerable variation in the length of the glands. Although the

caecum was considerably smaller than a normally filled one, it was by no means empty. The caecum and first part of the ascending colon have greater diameters than the remaining portions of the large intestine and their glands are shorter. In table 2, because of the existence of large mucosal folds, the perimeters, measured at the bases of the glands, are given along with the diameters.

TABLE 2

Measurements made from normally contracted pieces of large intestine of the guinea-pig. Total length of large intestine about 72 cm.

PORTION OF LARGE INTESTINE	MEAN DIAMETER	PERIMETER	DEPTH OF GLANDS
	<i>mm</i>	<i>mm</i>	<i>mm</i>
Caecum.....			0.13 to 0.18
Just below colic valve.....	3.7	24.0	0.18
30 cm. below valve.....	1.8	7.2	0.16 to 0.27
60 cm. below valve.....	1.4	6.1	0.18 to 0.27

The pieces of intestine described in these tables perhaps do not represent *normally contracted* intestine. They are emptied portions of the intestine which are further contracted owing to their immersion in Zenker's fluid. Whether or not under normal conditions the intestine ever contracts to the same extent is a difficult problem to solve (owing to the introduction of such factors as anesthesia, mechanical and chemical stimuli, etc.), the determination of which seems not to fall under the scope of the present work. It would perhaps be better, therefore, to use the term 'strongly contracted' in place of 'normally contracted.'

Normal distention

A guinea-pig weighing 565 grams was killed under normal feeding conditions. The stomach, caecum, and portions of the small and large intestines (especially the ileum) were greatly distended with food. Certain pieces of the intestine (2 to 3 cm. long) were tied off at both ends before removing, so as to retain the intestinal contents and so that the walls could not contract. An examination of cross and longitudinal sections

of such distended small intestine shows that the villi are shorter and broader than those of the contracted intestine (compare figs. 5 and 6). They present a variety of shapes; some are bent over on their sides, and others shortened on themselves so that their epithelium is thrown into wrinkles which appear twisted spirally. Central lacteals, as well as other lymphatic vessels, are enlarged. The glands are shorter and broader. Goblet cells appear about as numerous in the distended condition as

TABLE 3

Measurements showing the effects of normal distention of the small intestine as contrasted to those of contraction

CONDITION OF INTESTINE	REGION	DIAMETER	THICKNESS ¹ OF MUCOSA ¹	THICKNESS ² OF OUTER COATS ³	VILLI		GLANDS	
					Height ⁴	Breadth ²	Depth ⁵	Breadth
		mm.	mm.	mm.	mm.	mm.	mm.	mm.
Contracted	40 cm. below valve	1.5	0.63	0.18	0.58	0.08	0.22	0.04
Distended	55 cm. below valve	5.8	0.45	0.07	0.36	0.14	0.09	0.06

¹ Not including muscularis mucosae

² Average

³ Including muscularis mucosae

⁴ Highest

⁵ Deepest

in the contracted. In table 3 measurements from both the normally contracted and the normally distended intestines are given.

The large intestine in the filled condition also shows a marked shortening of its glands, as can be seen by comparing figures 8 and 9.

Artificial distention

Some of the results of artificial distention produced by pressure are given in table 4. As would be expected, different amounts of distention produce different degrees of shortening

TABLE 4

Measurements showing the effects of normal distention of the large intestine as contrasted to those of contracted

CONDITION OF INTESTINE	REGION	DIAMETER (mean)	GLANDS		THICKNESS OF OUTER COATS ²
			Depth ¹	Breadth ²	
		<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Contracted	30 cm. below valve	3.7	0.27	0.045	0.32
Distended	45 cm. below valve	6.4	0.13	0.062	0.06

¹ In this case the thickness of the glands is equal to the thickness of the mucosa; muscularis mucosae omitted; depth longest.

² Average.

of the glands and villi. In figure 2 is shown a section of the small intestine of a newborn guinea-pig distended by means of a column of water 150 cm. high. The glands throughout the whole piece of intestine have practically disappeared. In an adult guinea-pig, however, stronger distention (pressure about 270 cm. water) did not do away with glands to so great an extent although in some places they are entirely gone. Those few which remain are short and very broad. Distention of the large intestine (pressure of 150 c.m. of water) reduces the length of the glands considerably. They were further shortened by a pressure of about 270 cm. of water, but did not entirely disappear.

The epithelium also shows the effects of strongly distending the small and large intestines. Whereas in the contracted intestine the epithelium is composed of tall cylindrical cells, with rounded or elongated nuclei, in the strongly distended intestine it is much flatter, and is composed of cuboidal cells with nuclei which are also flattened. In figures 10 and 11 are shown the effects of strong distention of the large intestine upon its epithelium. Similar pictures may be obtained from the small intestine.

Contraction following artificial distention

A piece of small intestine was distended at a pressure of 150 cm. of water with warm normal salt solution. The pressure was maintained for about one minute and was then removed and placed immediately in Zenker's fluid. Contraction began in a few seconds time. In figure 3 is seen an empty piece of intestine taken from the region adjacent to the above. The distended and subsequently contracted intestine is shown in figure 4. Figure 7 shows the effects of a pressure of 150 cm. of water upon an adjacent piece of small intestine. Similar results were obtained with the large intestine.

THE INTESTINES OF OTHER ANIMALS

Cat. Cross sections of the empty small intestine of the cat fixed in Zenker's fluid show five to six low longitudinal folds of the mucous membrane. On these folds both the villi and the glands are longer and broader than those in the spaces between them, suggesting that the folds are regions of more strongly contracted mucosa. In the normally expanded intestine, however, the villi and glands are everywhere more uniform in size. The effects of distention, both normal and with a pressure of 150 cm. of water are shown in table 5.

TABLE 5

Measurements showing the effects of distention of the small intestine of the cat upon the shape of villi and glands

CONDITION OF INTESTINE	DIAMETER OF EP. TUBE	VILLI		GLANDS	
		Height	Breadth	Depth	Breadth
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Normally contracted.....	5.8	1.06	0.13	0.80	0.045
Normally distended.....	8.0	0.74	0.16	0.42	0.060
Experimentally distended.....	10.0	0.69	0.18	0.37	0.061

In the large intestine of the cat the glands are likewise shortened and broadened during distention as shown in table 6.

Dog. Artificial distention on the dog's intestine under a pressure of 7 pounds per square inch (bursting point, 9 pounds)

TABLE 6

Measurements showing the effects of distention of the large intestine of the cat upon the shape of glands

CONDITION OF INTESTINE	DIAMETER OF EP. TUBE	GLANDS	
		Depth	Breadth
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Normally contracted.....	11.5	0.45	0.056
Normally distended.....	15.0	0.27	0.070

did not result in a complete disappearance of either glands or villi. The effects were largely similar to those on the villi and glands of the cat.

TABLE 7

Measurements showing the effects of distention upon the small intestine of the dog upon the shape of villi and glands

CONDITION OF INTESTINE	VILLI		GLANDS	
	Height	Breadth	Depth	Breadth
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Contracted.....	1.26	0.22	0.81	0.03
Distended.....	0.40	0.32	0.13	0.07

Mouse. Experimental distention of the mouse's small intestine at a pressure of 100 cm. of water caused a greater shortening of glands than of villi. The appearance obtained is somewhat similar to that produced by distending the intestine of the guinea-pig, except that the villi of the mouse are relatively taller and are not folded on themselves. Table 8 shows the amount of shortening produced.

TABLE 8

Measurements showing the effects of distention of the small intestine of the mouse upon the shape of villi and glands

CONDITION OF INTESTINE	VILLI		GLANDS	
	Height	Breadth	Depth	Breadth
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Contracted.....	0.18	0.09	0.11	0.04
Distended.....	0.11	0.18	0.04	0.07

Distention of the stomach and oesophagus

Before concluding the present work it seems desirable to call attention to the effects of distention upon the mucous membranes of the stomach and oesophagus. Strong distention of the stomach of the cat and of the guinea-pig brings about a thinning out of the mucosa, a shortening of both pits and glands which at the same time are widened and spread apart.

In the oesophagus of these animals strong distention brings about a marked flattening of the stratified squamous epithelium and an apparent reduction in the number of its cell layers. Thus, the oesophageal epithelium of the cat which is normally composed of 13 to 18 layers of polygonal cells, on strong distention appears to consist of 6 to 8 layers of very much flattened cells. The effects here upon the epithelium of the oesophagus are somewhat comparable to those which may be produced upon the epithelium of the ureter through distention as described by Harvey ('09).

CONCLUSIONS

The effects of distention of the intestine may be enumerated as follows:

1. The outer intestinal coats become reduced in thickness.
2. The mucosa becomes reduced in thickness.
3. The villi become shorter and broader.
4. Glands become shorter and broader. In the guinea-pig and mouse they may entirely disappear if the intestine is strongly distended.
5. In the intestine of the guinea-pig the epithelium becomes flattened upon strong distention.

It is evident from the foregoing results that the shapes of villi and glands are to a great extent dependent upon the condition of distention or contraction of the intestine. This is true not only for marked distention produced experimentally, but for the smaller amounts of distention which take place under normal conditions. It seems probable, therefore, that with each dilation and contraction of normal peristalsis and

of the rhythmical movements of the intestine, the villi change their shape, and in this way bring about a more thorough mixing of the intestinal contents. Moreover, by the unfolding of the intestinal glands, a greater amount of epithelium is exposed to the intestinal content, and thus the absorption surface is increased. Because of the variety of shapes presented by the gland cavities it is not possible, by ordinary methods, to determine their exact capacities, but it is probable, that the capacities of the glands are decreased upon strong distention, and their contents are then partially emptied into the lumen of the intestine.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Normally contracted small intestine of a newborn guinea-pig. $\times 80$.
- 2 Small intestine of newborn guinea-pig distended with 150 cm. water pressure. $\times 80$.
- 3 Normally contracted small intestine of adult guinea-pig. $\times 80$.
- 4 Small intestine of same guinea-pig subsequently contracted after distention similar to that in figure 7. $\times 80$.

PLATE 2

EXPLANATION OF FIGURES

- 5 Strongly contracted small intestine of adult guinea-pig. $\times 80$.
- 6 Small intestine of adult guinea-pig normally distended with food material. $\times 80$.
- 7 Small intestine of adult guinea-pig distended with 150 cm. of water pressure. $\times 80$.

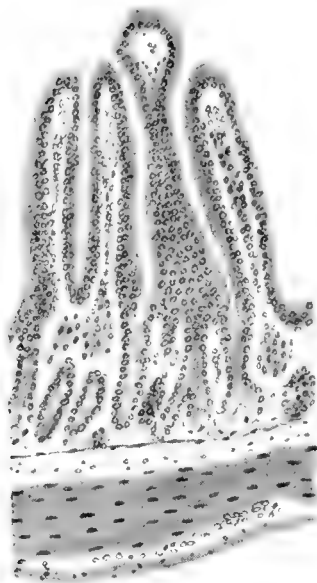


Fig. 1

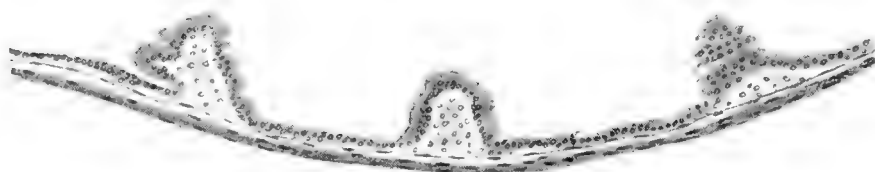


Fig. 2

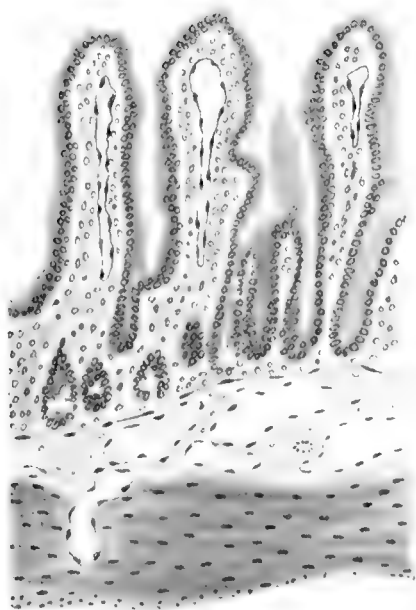


Fig. 3

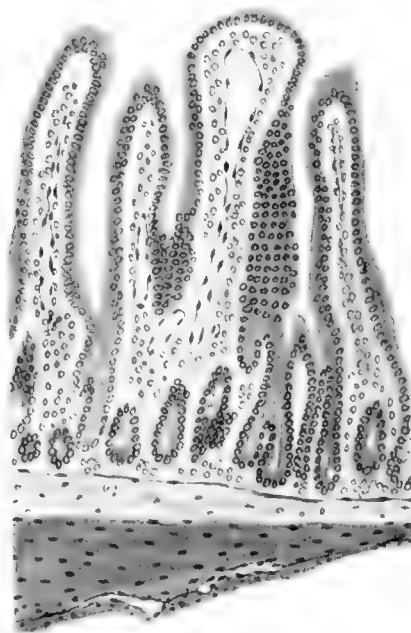


Fig. 4

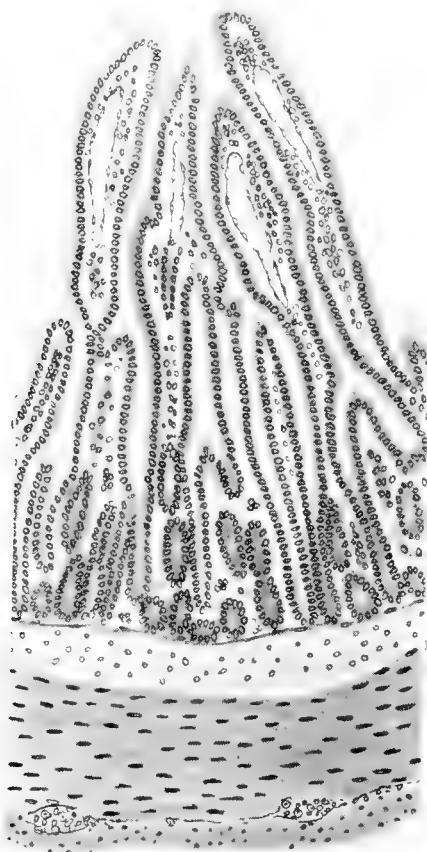


Fig. 5

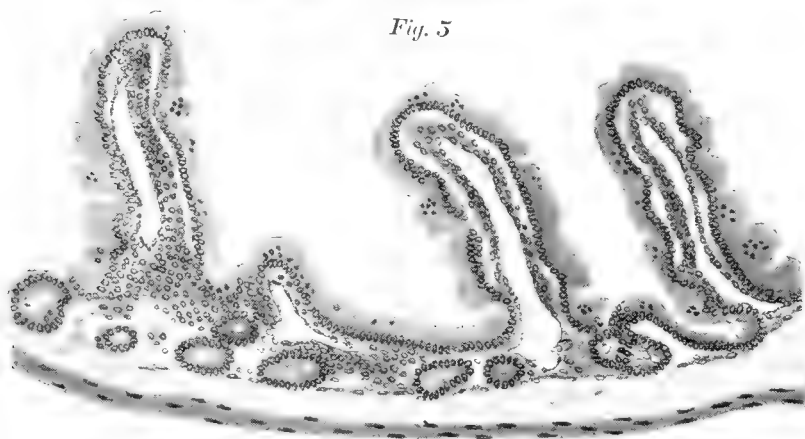


Fig. 6

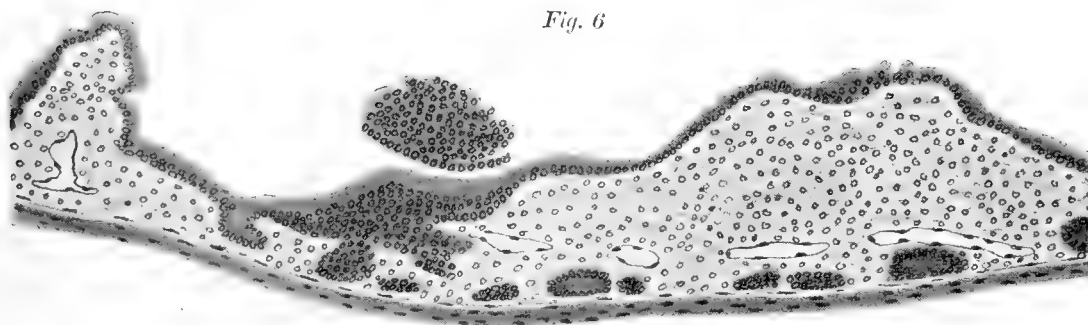


Fig. 7

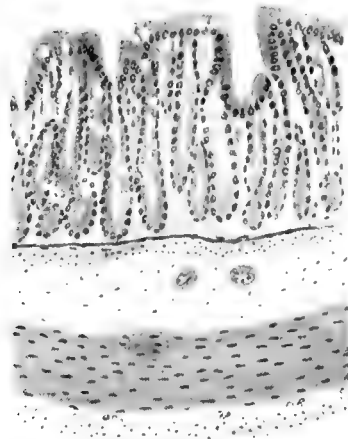


Fig. 8



Fig. 9

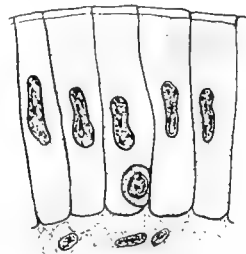


Fig. 10

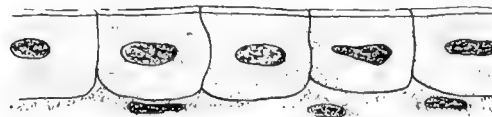


Fig. 11

EXPLANATION OF FIGURES

- 8 Normally contracted large intestine of adult guinea-pig. $\times 80$.
- 9 Normally distended large intestine of adult guinea-pig; (distended with gas). $\times 80$.
- 10 Surface epithelium of normally contracted large intestine of adult guinea-pig. $\times 720$.
- 11 Surface epithelium of greatly distended large intestine of adult guinea-pig. $\times 720$.

HISTOLOGY OF THE SENSORY GANGLIA OF BIRDS¹

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FORTY FIGURES

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¹ Contribution from the Zoological Laboratory of Northwestern University under the direction of William A. Locy.

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I. INTRODUCTION

The introduction of the modified silver reduction method of Cajal ('05) gave a new impulse to the study of the structure of the sensory ganglia. In these investigations, however, the ganglia of birds have received scant attention. Moreover, the observations have been confined chiefly to the spinal ganglia, with occasional references to the Gasserian, and scarcely any study of the remaining cerebral ganglia. It was with a view of supplying a more comprehensive histological analysis of the sensory ganglia of birds that this investigation was undertaken. The observations were carried on during 1909-10 and 1910-11 in the Zoological Laboratory of Northwestern University under the direction of Prof. William A. Locy, to whom grateful acknowledgment is accorded for assistance and supervision.

II. BRIEF COMMENTS ON THE LITERATURE

The more important steps in the development of knowledge regarding the nerve cells of the sensory ganglia may be summarily stated.² After the pioneer observation (Ehrenberg '33, Kölliker '44, etc.) came the recognition that the typical ganglion cell of adult vertebrates above the fishes, consists of a cell body with a main process that divides into two unequal branches, one going centrally and the other peripherally. Further studies showed that this typical form is produced by modifications of the oppositely polar cells of embryonic stages, and soon a whole series of transi-

² The pertinent literature (see bibliographical list) dealing with the histology of the sensory ganglia of all classes of vertebrates was read and considered, but would require too much space for a detailed review.

tional forms, from oppositi-polar to unipolar, was described and figured. The bipolar elements were shown to persist in large numbers in the sensory ganglia of lower vertebrates and the unipolar derivatives to be characteristic of the ganglia of mature higher vertebrates.

His ('86) first made known the condition in the human embryo by finding the cells of the spinal ganglia to be bipolar up to the ninth week of development and, thereafter, gradually to change into unipolar cells with a *T*-shaped process.

Multipolar cells now began to be described in sensory ganglia as: Disse ('93) frog; Cajal ('93) and von Lenhossék ('94) in spinal ganglia of chick embryos; Spirlas ('96) goat embryos; Dogiel ('96) adult mammals. Huber, also, in 1896, described a new kind (spinal ganglia of frog, turtle, and embryo chick) with accessory processes springing from the cell-body and terminating, frequently, with bulb-like enlargements, within the capsule. He suggested that multipolar cells in sensory ganglia are, probably, from the sympathetic.

Passing over much detail, the next general step was the identification of numerous types of cells in the sensory ganglia of mammals. Dogiel in 1896-97 described a new variety of cell (type II) with fine processes producing arborizations within the ganglion, but without a distinct axon. Lugaro ('94) indicated five distinct types, and in 1908 Dogiel made an elaborate classification embracing eleven positive types and intermediate gradations. The eight morphological varieties of Cajal ('06) are more easily recognizable than the types of Dogiel.

Turning now from vertebrates in general to researches on the sensory ganglia of birds we find eleven publications, of which only the salient points will be indicated.

Retzius ('80) found two kinds of unipolar cells in the spinal ganglia of the chick; one with, and one without the medullated processes. Both kinds were without glomeruli. The nerve elements of the Gasserian were found to be essentially similar to those of the spinal.

Rawitz ('82) in a comparative study of the ganglia of all classes of vertebrates, observed the structure of the spinal and Gasserian

ganglia of seven kinds of birds. In the raven, goldfinch, and cardinal bird he describes finely granular unipolar cells of variable shape and two varieties of nerve fibers, large and small, the coarse fibers running through the ganglion without being connected with the ganglion cells. In the sensory ganglia of the chick only coarsely granular unipolar cells were observed surrounded by tough pericellular tissue. In swimming birds (duck and goose) the cells were bullet-shaped, unipolar, and very finely granular.

van Gehuchten ('92 a and '92 b) investigated conditions in embryos of the duck and the chick. The cells were oppositipolar up to the twelfth day of incubation. In twenty-day embryos he found oppositipolar, unipolar, and transitional stages. In the adults he declares all cells to be unipolar. He found similar conditions in ganglia of the fifth, the ninth, and the tenth.

The observations of von Lenhossék ('92) indicate that the cells of the sensory ganglia of the seven-day chick are all bipolar, and ('94) that the first tendency toward unipolarization is seen on the ninth day; by the fourteenth to fifteenth days of incubation unipolar cells are numerous. Cajal ('93) found multipolar cells in the spinal ganglia of the embryo chick and concluded that they later develop into those of the ordinary type.

Huber ('96) observed cells with accessory processes in the dorsal spinal ganglia of the chick. These are not of frequent occurrence.

Timofeew ('98) describes chiefly the internal structure of sensory ganglia showing a fibrillar reticulum of the cell body. He found small tigroid bodies, with fibers that form networks, to be characteristic of spinal and sympathetic ganglia of birds. The cells range from 8 to 40μ in diameter. Two nucleoli were regularly seen in each nucleus, one of which took an acid and the other a basic stain.

Levi ('05, '06 and '08) made observations on the spinal ganglia of pigeon embryos and found bipolar spindle shaped cells with three processes. In the later stages, when the cells become unipolar, the third process appears to unite with the axis cylinder and become a part of it. In other bipolar cells there issued fine collaterals from the two processes. He could not confirm their

presence in the adult. In 1906, he described lobed cells in cerebro-spinal ganglia of the tenth (see my observations on the owl). In the same ganglia he found a few fenestration cells, also a small number with accessory processes and delicate collaterals on both processes. In his extensive paper of 1908 he deals with the histology and histogenesis of cerebro-spinal ganglia in all classes of vertebrates. That part devoted to the ganglia of birds is relatively brief. It includes observations on the Gasserian (semi-lunare) and vagus (plessiforme) ganglia of the adult hen, and the adult domestic duck, also the spinal ganglia of the duck, and sensory ganglia of the six-day chick and of the six-day duck embryos. In the Gasserian of the hen he finds the cells of the usual type, but, in the vagus of the same bird he found oppositipolar cells predominating and only a few transition stages between that condition and unipolar. In the vagus of the duck, conditions were reversed, most of the cells were unipolar with *T* and *Y* branching processes, and bipolars were infrequent. In duck and chick embryos he noted some details as to variation in the cells, but found in the younger stages oppositipolar cells, and in older individuals transitional stages and a few unipolars.

III. OBSERVATIONS

The material used in my observations consisted of the spinal, ninth, tenth and Gasserian ganglia of the chick; the spinal, eighth, tenth and Gasserian of the screech owl; the spinal, ninth, tenth and Gasserian of the goose, of the duck and of the pigeon respectively; the spinal and Gasserian of the turkey; and the spinal of the sparrow.

In addition to my own preparations I had the use of serial sections of the chick and duck prepared by the Cajal method by Miss Enid Hennessey.

Of the fixatives employed two were used extensively, ammoniacal alcohol and 5 per cent formalin. In the alcoholic fixative the amount of ammonium hydroxide was varied, from a few drops in 50 cc. of absolute alcohol, to 5 cc. of the ammonium hydroxide in 100 cc. of 95 per cent alcohol. The best results were obtained from material fixed in the 95 per cent alcohol with 5 per cent

ammonium hydroxide. The material thus fixed was stained by the silver reduction method of Ramón y Cajal (see "Ergebnisse der Anatomie und Entwicklungsgeschichte," vol. 16, page 178). Some material that did not stain well by the silver reduction method was counterstained with Delafield's hematoxylin which colored the nerve fibers and capsular nuclei intensely and the Schwann's sheath very delicately.

Material fixed in formalin gave good results as regards the capsule, capsular nuclei and the nuclei of the sensory cells, while material fixed in ammoniacal alcohol gave good pictures of cell outline, cell structure, and nerve processes.

If preserved for any length of time in alcohol the material depreciated. The best results were obtained from material that was carried through the fixing and staining processes without delay.

In the dorsal region, the sympathetic and sensory ganglia are so united that it is difficult to separate them, accordingly they were treated and sectioned together. In almost every instance the sympathetic ganglia took the silver stain readily, but cells of the sensory ganglia were more refractory. This made it necessary to employ a large number of preparations. The ganglia of old birds stained more readily than those of the younger birds.

A. SENSORY GANGLIA OF THE CHICK

There are several structural features that are common to the nerve cells of the different ganglia of the chick and other birds—as individual variation in shape, and in position of nuclei, the surrounding capsule, etc.—that need not be separately described for each ganglion. The predominating type of unipolar cell, with unequal branches of the main process, is the result of modification. In embryonic stages the nerve-cells start as bipolar (usually oppositi-polar) cells and change into the unipolar condition. In the chick of eleven to fourteen days incubation the process of change is well under way and numerous gradations are to be seen. In adult birds most of the ganglion cells are in the unipolar stage but there remains about 2 per cent of bipolar cells in various stages of transition.

1. *Spinal ganglia*

In spinal ganglia, the nerve cells at the periphery lie close together, while in the central part they are separated into groups of 4 to 8 cells by bundles of processes, the fibers from the peripheral cells uniting with those from the central cells to form the separating bundles. Within the groups the cells are arranged in single rows lying parallel to the long axes of the ganglia. The cells are also slightly closer together at the proximal than at the distal end because the centrally directed processes are uniformly smaller than those peripherally directed.

The spinal ganglion cells of the chick vary in size, the major diameters ranging between 22 and 75 μ and the minor between 18 and 40 μ . There is also a variation in the shape of the cells, the more frequent forms being rounded (figs. 1, 4), elliptical (figs. 6, 7), and pear-shaped (fig. 3), but others in close contact with surrounding cells are irregularly polyhedral.

The nuclei of the ganglion cells are sharply defined and vary from round to elliptical in shape. The rounded average about 8 μ in diameter and the elliptical average 9 to 10 μ in length and 8 μ in breadth. The nucleus is usually centrally located (figs. 1, 4), but in some cases it is close to the cell wall (fig. 3). The long diameter of the nucleus while generally parallel to the major axis of the cell, is sometimes at right angles to it (fig. 5). The larger nuclei are found in the larger cells, but measurements show that the size of the nucleus does not vary in proportion to the increase in the size of the cell. A single centrally located nucleolus is usually to be seen.

Each cell is enclosed in a thin connective-tissue capsule or mantle that extends a greater or less distance along the process (figs. 1, 6). A considerable space separates this mantle from the wall of the cell. The round to elliptical capsular nuclei vary in size and contain one or two centrally located nucleoli.

About 98 per cent of the spinal ganglion cells of the adult chick are unipolar and the processes mostly without glomeruli. A small percentage of the processes had an initial glomerulus of simple character (fig. 7) showing one of the most complicated

observed. Clearly defined implantation cones (figs. 1, 4, 6) are infrequent. The main process is relatively broad as it emerges from the cell, but soon narrows and preserves a uniform width until it branches. It usually springs from the distal pole of the cell and is directed distally and also toward the central longitudinal axis of the ganglion. The process of the unipolar cell divides into two unequal branches (fig 1) at variable distances from the cells, the larger branch going peripherally and the smaller centrally. One process was followed for 192 μ without the division having taken place.

The few bipolar cells exhibit various transitional stages from the oppositi-polar to the unipolar (figs. 2 to 6). As in unipolar cells, so in bipolar, the distally directed process is larger than the proximally directed.

A limited number of small protoplasmic slings and fenestrations were observed in the nerve cells of the spinal ganglia (fig. 8, *fen., slg.*).

2. Cerebral ganglia

a. Ganglion of the ninth nerve. The glossopharyngeal ganglion of the chick is less than 0.5 mm. in diameter and difficult to free from the connective tissue. A median, longitudinal section of this ganglion (fig. 12) shows that the cells are more numerous at the center than at the periphery, and that areas of considerable extent lack cells entirely. Here also, the prevailing type of cell is unipolar with the processes of unequal size, the smaller being directed centrally. Many non-medullated fibers occur in this ganglion. In shape the cells are rounded to elliptical, the elongated forms being the more numerous. The long axis of the nerve cells is in almost all cases parallel to the long axis of the ganglion. The cells are noticeably smaller in this ganglion than those in the other ganglia of the same bird, their major diameters varying between 20 and 50 μ , and their minor diameters between 16 and 33 μ . The round to elliptical nuclei are also relatively smaller than in cells from other ganglia and only one nucleolus was observed in any nucleus. A few bipolar cells were observed

exhibiting stages between the oppositi-polar and the unipolar (figs 12, b_1 , b_2). Implantation cones were infrequent.

b. The vagus ganglion. The ganglion of the tenth nerve is so encased in bone that it is difficult to remove it without injury. This large, complex ganglion is, in the adult fowl, about 2 mm. long by 1.25 mm. broad, and it must be looked upon as the product of the union of several ganglia. The roots of the glossopharyngeal run through the vagus. Sections of the vagus, embracing also the roots of the ninth nerve, show cells in that part through which the fibers of the ninth run, but it was impossible to determine whether any of these cells send fibers into the ninth nerve. The cells are more numerous around the periphery than in the center of the ganglion where they are separated into elongated groups by bundles of fibers. At the proximal end the large number of fibers passing into the numerous roots give to this part of the ganglion a characteristic appearance.

In the vagus ganglion there is considerable range in the size of the cells (fig. 13, *b* and *c*), the major diameters varying between 18 and 74 μ and the minor between 15 and 40 μ , those of medium size predominating. Cells of unusual length are generally narrow. The shape of the cells differs as in the other ganglia, round, elliptical, and pear-shaped being common while a small number are club-shaped. The nuclei are round to oval, the rounded ones being from 6 to 11 μ in diameter and the oval ones between 6 and 16 μ in length. A single nucleolus located near the center was observed in each nucleus.

As in the other ganglia the prevailing type of cell is unipolar. There were a few bipolar cells showing gradations between oppositi-polar and unipolar. In a careful count of ten conservative sections of a vagus ganglion of a six-year-old hen were found 481 unipolar cells and only 2 bipolar; 57 of the cells showed pericellular networks, 5 of them had accessory processes and 3 showed fenestrations. In two other sections of the same ganglion, out of 114 unipolar cells, the processes of 101 issued from the distal end of the cell and continued in a distal direction. In one instance only the process issued from the proximal end, and the processes of the remaining 12 cells emerged from the side of the cells. The major-

ity of those issuing from the sides of the cells turned distally after a very short course. In these counts few implantation cones (fig. 13, *impl.c.*) and few (2 out of 481 cells) initial glomeruli were seen.

The ganglion cells of the old hen and of the spring chicken showed interesting differences. While in the old hen, the periphery of the cells was shrunken and crinkled, in young birds, the cells were more uniformly rounded and plump (see also under Gasserian ganglion). Few fenestrations and protoplasmic slings were observed in the young bird but were not uncommon in the old hen. Figure 9 shows a cell of the vagus ganglion of the old bird with fenestrations (*fen.*) and small protoplasmic slings (*slg.*). Surrounding this cell there is a pericellular network (*pr.cl.*).

The pericellular fibrous structures in this ganglion are of two kinds. One is a true network in which fibers branch and anastomose (figs. 10 and 14, *pr.cl.*). The other is shown in figure 10, *b*; it is not a true net, but like a thread running several times around the cell, from which fibers extend into the intercellular space (fig. 10, *fbr.*). The pericellular networks do not come in contact with the cell but lie close to the capsule.

Bundles of non-medullated ('afferent') fibers, probably from the sympathetic system, ramify among the cells of the sensory ganglia. Figure 13 fairly represents the situation. This is a sketch of part of a section from the peripheral region of a vagus ganglion of the old hen. The large bundle (*L*) ran into one of the roots of the vagus; it showed in four consecutive sections and, accordingly, was not less than 36μ in thickness. The figure shows the cut ends of the larger number of these fibers as they curved upward to pass over the cells that lie in their path. Some of the non-medullated fibers joined the irregular network about the cell marked *b*. A number of the fibers of the intercellular space were also connected with this network. The network extended about the process but was entirely confined within the capsule.

c. The Gasserian ganglion. The Gasserian is the largest of the sensory ganglia found in the chick, and shows marked complexity in the arrangement of its elements. Large bundles of fibers pass through the ganglion without being connected with the

nerve cells. The cells of the ganglion are irregularly grouped as in the spinal and tenth ganglia. They are more crowded at the periphery than at the center. In the center the bundles of nerve processes separate the cells into groups of varying numbers, so arranged that the long axes of the groups are parallel to the long axes of the ganglion. The cells are more numerous at the proximal than at the distal end of the ganglion, this being a reversal of the condition found in the ganglion of the tenth nerve. The sensory cells range in size from those with the major axes between 25 and 70 μ , and minor diameters between 15 and 40 μ . The nuclei are uniformly regular in shape, varying from round to elliptical. The major diameter of the nuclei is between 8 and 14 μ and the minor diameter is between 6 and 11 μ . A single centrally placed nucleolus was observed in each nucleus.

Good examples of perinuclear network were abundant in the cells of the Gasserian ganglion. The meshes of the network are finer near the nucleus, and, since these fibrillae stain deeper than the remaining substance of the cell, the close meshed perinuclear network appeared as a dark ring around the nucleus.

A notable coiling of the central axis, not involving the sheath, was observed in a Gasserian ganglion counterstained with Delafield's haematoxylin. Fibers of this nature were in tracts free from cells and also in parts of the ganglion containing cells. Figure 11 shows this condition; the two sections of the picture represent a single process, the ends *a* and *b* being continuous. A reëxamination of other Gasserian ganglia, not counterstained, showed the presence of many fibers having similar convolutions. It would appear that this condition is not uncommon, though I have found no recognition of it in the literature.

The Gasserian ganglion of the old hen presents histological features that differ widely from those observed in the younger birds (see also under Vagus). The regularity of outline in the cells, so marked in the younger birds, is not characteristic of the old hen. The outline of the cells of the old bird is frequently quite irregular and the surface presents numerous rugosities.

Vacuoles, varying in number from one to six, are also of frequent occurrence in the cells of the Gasserian of the old hen.

In size they range from 3 or 4 μ in diameter to those that occupy nearly half of the cell. Figure 17 shows a large vacuole that occupied nearly half of the area of the cell. This cell contained two other vacuoles of much smaller size. In this instance the vacuolated condition could scarcely be due to fatigue since the hen was caught early in the morning, and had no opportunity to exercise between the time of its capture and the time of its death. The hen was at least six years old and in a normally healthy condition.

In two typical sections of the Gasserian of the old hen there were 99 unipolar cells of which 2 had simple initial glomeruli. Among the 99 were 5 bipolar cells showing stages from oppositipolar to unipolar; 7 cells had accessory processes, 20 had sling processes, and there were 46 cells containing vacuoles.

Accessory processes were present and varied in size from those visible only with the highest power of the microscope to those readily seen with the low power. These accessory processes usually terminated in small bulbs and none were observed to extend beyond the pericellular capsule (fig. 19). Occasionally a cell was observed in which minute processes with pin-head terminations issued from the main process near its junction with the cell.

The fenestrations of cells in the Gasserian ganglion varied from small perforations of the periphery of the cell, to an extensive network of anastomosing processes occupying more than half of the space within the capsule. Figure 20 (*fen.*) shows one of the simplest fenestrations consisting of a single perforation at the base of the slender centrally going process. An accessory process arises near the fenestration. In fig. 21 there are protoplasmic slings (*slg.*) at the base of the process and also at the opposite pole. Although extending some distance from the cell wall, they do not pass beyond the capsule. Figure 22 represents a cell in which the loops of protoplasm are near or associated with the main process. In figure 23 there are protoplasmic loops on the pole opposite the main process. The protoplasmic strands diminish in size as they recede from the cell body so that near the capsule the fibers become very fine. In this case the body of the cell occupies about one-third of the space within the capsule.

Cells with the fenestrations, slings, and loops, while quite common in the old hen, are comparatively rare in young birds. Figure 24 represents a bipolar cell from a young bird showing a similar though less complicated condition.

The question of the presence of multipolar cells and of their nature is an important one from the standpoint of the literature. Accordingly, a careful search was made for such cells in the sensory ganglia of the chick. The Gasserian, glossopharyngeal, vagus, the cervical, dorsal and lumbar spinals were analyzed without finding any cell that should be properly classified as multipolar. There is a clearly defined difference between multipolar cells as observed in the sympathetic ganglia of the chick, and the cells with accessory processes of the sensory ganglia. A comparison of figures 18 and 19 will illustrate this difference. In figure 19 the accessory processes are very fine as compared with the neuraxon, they are entirely included within the capsules, and, in the majority of instances, each ends in a bulb. In figure 18, which is a sympathetic ganglion cell, the processes that pass beyond the capsule are large and of fairly uniform size. In the intercellular space they divide into numerous branches. Moreover this cell (fig. 18) has slings and accessory processes, confined within the capsule, that are similar to those of sensory cells. It appears, therefore, that the supernumary processes that make the sympathetic a 'multipolar' cell are not present in sensory cells, while the sympathetic type of cell has accessory processes similar to those found on the cells of sensory ganglia and in addition, has its supernumary processes extending beyond the capsule.

The principal sympathetic ganglion in close proximity to a cerebral ganglion lies just beneath the vagus. It can, however, be readily separated from that ganglion. In the cervical region, also, the spinal and sympathetic ganglia are separate, but the dorsal spinals are closely united with the sympathetic. Sections of the dorsal spinal ganglia show cells of sympathetic type close to those of sensory type. The space between sympathetic cells and those of truly sensory type is often less than the diameter of an average sensory cell, and their capsules appear in some instances to touch. Thus it is easy to account for the multipolar

cells of sympathetic type in sections of sensory ganglia. Careful search, however, did not show the presence of sympathetic cells on the sensory side of the ganglion. There is proximity without actual mixture of the cells. Non-medullated fibers in large numbers crossed from the sympathetic to the sensory sides.

B. SENSORY GANGLIA OF THE OWL

1 *Spinal ganglia*

There is considerable variation in the size of the spinal ganglia of the owl; those of the brachial and lumbo-sacral regions are relatively large, being about the size of the Gasserian, and the cervical and the remaining dorsal and lumbar are small. In the large ganglia the cells are more numerous at the periphery than at the center, but lack sympathetic arrangement. In the smaller ganglia the cells are closely packed and are not divided into groups.

The cells of the spinal ganglia of the owl, although considerably smaller, conform closely in shape to those of the chick. They are for the most part rounded to elliptical and embrace only a limited number of forms that are so characteristic of the Gasserian and vagus of the same bird. The major diameters vary between 16 and 50 μ , and the minor between 8 and 27 μ . Large cells occur infrequently with major diameters above 45 μ and minor above 25 μ . In contrast with these conspicuously large cells are present small cells, some of them not more than 16 μ long and 8 μ wide. While there are gradations between the largest and the smallest, most of the cells are of a medium size with long diameters of 20 to 27 μ and short diameters of 18 to 22 μ .

The centrally located nuclei are round to elliptical and vary in major diameter between 5 and 10 μ , and between 4 and 8 μ in minor diameter. The smallest nucleus measured was 4 by 5 μ and the largest 8 by 9.

The ganglia of the brachial region show two classes of cells, the division being based on size. Those of the larger size constituted about 20 per cent of the entire number of cells in the ganglion, and had for their average major diameter 34 μ , and for

average minor diameter 21μ , while those of small size, including 80 per cent of the cells, had an average major diameter of 14.5μ and an average minor diameter of 12.5μ .

2. *Cerebral ganglia*

a. The vagus ganglion. In the screech owl this ganglion is slightly smaller than the Gasserian. Near the periphery the cells are compactly arranged, but at the center are distributed in clusters that are separated by bundles of nerve fibers and connective tissue.

In the vagus ganglion of the owl the largest cell observed was 41μ long and 33 broad, and the smallest one 18 by 17μ . The average size is about 32μ long and 27 wide. The majority of the cells are rounded in outline, the cells with the major diameter much greater than the minor diameter being few in number. There are also lobulated cells, ranging from mere superficial irregularities to deep indentations forming distinct lobes (fig. 25). Figure 27 (*lb.*) shows a lobe of intermediate size.

The nuclei are very regular, with clearly defined outline. They are round to elliptical in shape but the rounded ones are much more numerous than the elliptical. The elliptically shaped nuclei are found in the cells that have considerable difference between their major and minor diameters. Only one nucleolus was observed in any one nucleus.

In cells of vagus and Gasserian twists and turns of the main process are common (figs. 29, 30, 32), and occasionally a simple glomerulus was seen (fig. 25, vagus; fig. 33, Gasserian).

Accessory processes arising from the main process near its junction with the cell are of frequent occurrence. These processes usually end in small rounded enlargements, but in some cases they end without enlargements. Figures 28 and 29 show minute accessory processes terminating in bulbs, while figure 25 shows accessory processes without and one with the enlarged termination. All the accessory processes observed (except *ac.pr.*, in fig. 30) terminated within the capsule.

b. The Gasserian ganglion. The cells of this ganglion in the screech owl are relatively smaller than those in the chick, and are arranged in elongated groups of three to five cells throughout the larger portion of the ganglion, only a narrow peripheral portion of the ganglion lacks this grouping of the cells.

The cells are more uniform in size than those of the Gasserian of the chick. The major diameters vary between 20 and 40 μ , and the minors between 15 and 30 μ . A small number of the cells are rounded, a larger number are elliptical to pear-shaped. Lobulated cells are characteristic and form a series from mere irregularities of outline (fig. 31) to well formed lobes (fig. 32).

Although the cells show such variations the nuclei are decidedly regular. They vary from round to elliptical, the rounded form predominating. They were usually near the center, but sometimes close to the cell wall. A single nucleolus was observed in each nucleus.

The processes do not follow a direct course near the cell as in the Gasserian of the chick. Quite commonly they take a meandering course (fig. 32) as also in the vagus of the same bird. Occasionally, an initial glomerulus is observed (fig. 33). As in the vagus ganglion, the process issues as frequently from the minor axis as from the major axis of the cell.

Accessory processes near the base of the main process (figs. 31, *ac.pr.*) were not uncommon. They were usually very fine and terminated in small rounded enlargements. Sometimes, also, they emerged from the main process near its place of union with the cell (fig. 31, *ac.pr.*¹). Few examples of fenestration (fig. 31, *fen.*) were seen.

Pericellular networks were observed in a few instances, being similar in structure to those described in the vagus ganglion of the chicken. In the Gasserian of the owl were seen, very distinctly, the non-medullated fibers so prominent in the Gasserian, tenth, and spinal ganglia of the chick. These fibers stain black and are about one-fourth the diameter of the main processes of the sensory cells. A few small vacuoles were observed.

c. Ganglion of the auditory nerve. The cells of this ganglion are relatively small and bipolar, the majority of them being

oppositi-polar (fig. 34). An occasional departure from the oppositi-polar condition occurs, but in no instance is there any near approach to the unipolar. The cells are nearly uniform as to shape and size, the shape being oval, with conical poles from which emerge the processes. The largest cells have a major diameter that averages $33\ \mu$ and a minor that averages 18, while the smaller cells have major diameters varying between 14 and $20\ \mu$ and minors varying between 10 and 12.

The nuclei are round to elliptical in shape. In the larger cells the major diameters of the nuclei vary between 5 and $8\ \mu$, while in the smaller cells the major diameter vary between 3 and $5\ \mu$.

The peripherally directed process was larger than the one centrally directed but there was less difference of size than in the branches of the sensory cells of the other ganglia. The processes for the most part followed a direct course as they issued from the cell, but as shown in figure 36, some of them exhibit an initial glomerulus of a single loop. These glomeruli were found as frequently on the centrally directed processes as on the distally directed ones.

C. SENSORY GANGLIA OF THE GOOSE

1. *Spinal ganglia*

The only spinal ganglia of the goose carefully studied were from the cervical region. These ganglia measure about 3 mm. in length and are large as compared with the corresponding ganglia of the other birds. The ganglion cells are distributed largely in clusters with a variable number of cells and separated by areas free from cells.

The cells vary considerably in size and shape. The majority are elliptical, some are rounded, others are irregular, and a few are elongated. One of the largest cells observed was 50 by $36\ \mu$, one of the smallest was 19 by 14. Some of the elongated ones varied much from the ordinary proportions, one of these having a major diameter of $74\ \mu$ and a minor diameter of only 16.

The predominating form of nucleus is elliptical, a smaller number only being rounded. They vary in major diameter between 8 and $15\ \mu$ and in minor between 5 and 13.

2. Cerebral ganglia

a. Ganglion of the ninth nerve. In the goose this ganglion is of large size when compared with that of the chick, the difference in size being due largely to the difference in size of the sensory cells rather than to a difference in the number of cells in the ganglion. A careful count of the number of cells was made in a medium longitudinal section of the ganglion of the goose, and in a corresponding section of the ganglion of the chick. The section from the goose ganglion showed 165 cells, that from the ganglion of the chick 160, so that the difference in size does not indicate a relatively larger number of cells. There is a segregation of the cells around the periphery of the ganglion, with irregular clusters of cells in the central portion and areas of considerable size which are devoid of cells.

The average size of the nerve cells of the ganglia is about 42 by 27 μ . Their major diameters vary between 30 and 69 μ and the minor between 19 and 41.

The nuclei vary from round to elliptical and are of considerable difference in size. Their longer diameters range between 8 and 14 μ and their shorter between 7 and 11. As observed in cells of other ganglia, the larger nuclei are in the larger cells, the long axis of the nucleus being usually parallel to the long axis of the cell.

The capsule lies close to the cell wall and the capsular nuclei are numerous and show distinctly. They are rounded to elliptical in outline and have one or two nucleoli. Similar nuclei are scattered rather thickly in the intercellular space and along the processes. Figure 37 represents a typical cell from this ganglion.

b. The vagus ganglion. In this large ganglionic mass the cells are distributed in elongated clusters, each cluster consisting, as a rule, of a single row of cells arranged with major diameters parallel to the long axis of the ganglion. The number of cells in each cluster is from 3 to 8. The more elongated cells are in greater number around the periphery of the ganglion, and those with major and minor diameters more nearly equal, are more abundant in the central portion. The cells are much more nu-

merous at the proximal than at the distal end. Nerve cells were found distributed for a considerable distance along the path of the tenth nerve, but in the ninth nerve no cells were found beyond the place of its emergence from the ganglionic mass.

Although cells were abundant in that part of the ganglionic mass through which the fibers of the ninth nerve passed, yet processes from these cells could not be traced into the ninth nerve, but many could be traced from the cells into the tenth nerve. Only a few cells in the vagus ganglion approached the rounded form. Nearly all were much longer than broad and the very large cells were frequently more than twice as long as broad. The prevailing shape is elliptical, but there were a number of club and pear-shaped forms. There were very few that showed much irregularity of outline, there being sufficient room for the cells without crowding. Figure 39 shows one of the greatest irregularities observed.

The ganglionic cells of the vagus of the goose are remarkable for their great size (fig. 39), being much larger than the cells seen in any other bird, larger even than those of the Gasserian ganglia of the same bird. The cells measured ranged in length between 33 and 102 μ , and in breadth between 25 and 48 μ . The average length of the cells is between 55 and 60 μ and the average breadth between 33 and 35.

The nuclei have clearly defined boundaries and are mostly round to elliptical. The larger cells have the larger nuclei, but the increase in the size of the nucleus is not proportional to the increase in the size of the cell. The largest nucleus observed measured 12 by 16 μ and the smallest 9 by 11.

The main process usually issues from one pole of the cell and is directed toward the distal end of the ganglion, but numerous exceptions were observed. The processes are large and emerge from the cell in a straight line or with but slight deviation from the straight course (fig. 39). No initial glomerulus was observed, but in one cell the process was quite twisted. Implantation cones were not observed.

The capsules of the cells were clearly pictured (fig. 39). They were quite delicate and had a number of clearly defined nuclei

each of which possessed one to two nucleoli. The capsular nuclei varied considerably in size and shape, some were round, others elongated. Outside the capsule, in the intercellular space, were scattered many similar nuclei. In a teased preparation more of the extra-capsular nuclei were spindle-shaped than round or elliptical. The capsular nuclei could be traced along the processes for a considerable distance after the process left the ganglion (fig. 39).

c. The Gasserian ganglion. This ganglion of the goose is very large, being 4.5 mm. long and over 3.5 mm. wide. The cells are more numerous about the periphery than in the central region. There is a slight tendency toward the grouping of cells into rows parallel to the course of the fibers, this tendency being somewhat more marked at the peripheral region than in the central. The cells are not crowded in this ganglion as in the ganglia of smaller birds, and are free from that irregularity of outline due to pressure.

The cells are of large size as compared with those of the chick and owl. They vary in major diameter between 20 and 70 μ , those of great length being usually narrower than those of average length. The minor diameters vary between 18 and 41 μ . A large percentage of the cells are rounded in shape, others elongated, and a small number are irregular.

The nuclei are round to elliptical, the round ones being in the rounded cells and the elliptical in those cells in which there is a considerable difference between the major and the minor diameters. The round nuclei are from 8 to 11 μ in diameter, the size of the nuclei varying somewhat as the size of the cell. The elliptical nuclei are between 11 and 16 μ long, and between 8 and 14 μ broad. The nuclei are usually near the center of the cell, but in a few cells they were located near the walls of the cells. The processes are relatively large, and, as in the cells of the vagus they follow a direct course. No initial glomerulus was observed.

The thin capsule of the cell is clearly defined, and encloses a considerable space between it and the wall of the contained cell. It is unlikely that this space is due to the shrinkage of the cell as might be supposed in the case of the chick. The ganglia of

the chick being fixed in absolute alcohol, shrinkage might be expected, but the ganglia of the goose were fixed in formalin which tends to increase rather than diminish the size of the cells. The capsular nuclei are quite numerous and lie not only in the pericellular capsule but also along the process (fig. 38). Somewhat similar nuclei are quite abundant in the intercellular space. Those in the capsule are usually elliptical with a major axis about twice the length of the minor, while those in the intercellular space are much broader, being round to elliptical. They contain one to two nucleoli. Figure 38 represents a typical cell of this ganglion showing the nucleus centrally located with its central nucleolus. The delicate sheath, *cap.*, with its elongated nuclei, *c*; are shown, also the nuclei, *nu*, of the intercellular space.

D. SENSORY GANGLIA OF THE DUCK

1. *Spinal ganglia*

The brachial ganglia of the mallard duck are much larger than any other spinal ganglia of the same bird. This condition is probably coördinated with their power of flight. As in the owl, the ganglion cells can be divided into two classes on the basis of their size. The large cells are fairly uniform in dimension; one of the larger ones measured 44 μ long and 41 broad, with a nucleus that had diameters of 14 and 12 μ respectively. The smaller cells, which are more numerous, average about 25 μ in length and 20 in breadth. One of the smallest of this class measured 11 by 18 μ and had a nucleus 4 by 6 μ . Outside of the brachial region the spinal ganglia of the mallard are much smaller and there is no division of the cells into two classes based on size. It is true that there are a few quite large cells and very many small ones, but there are numerous gradations between the extreme sizes.

One of the largest cells measured 71 μ in length, and 39 in breadth, with a nucleus 14 by 15 μ . One of the smallest was 14 μ in breadth and 15 in length, with a nucleus 4 by 5 μ .

In the brachial ganglia as well as in the other spinals most of the cells were rounded in shape, the remainder being elliptical

and irregular. Initial glomeruli were not observed, and implantation cones were seen only in spinal ganglia outside of the brachial region.

2. Cerebral ganglia

The cerebral ganglia observed were the ninth, tenth, and Gasserian of the common domestic duck and of the domesticated mallard.

a. Ganglion of the ninth nerve. The glossopharyngeal ganglion of the common domestic duck measured 1 mm. wide and the cells extended along the ganglion for 1.5 mm. The cells are much more numerous at the periphery than at the center, but they are not uniformly distributed at all parts of the periphery. One surface bulges into a pocket in which the cells are more numerous than at the opposite side. In the center the cells are comparatively few, and are divided into rows by the large bundles of fibers. In a section cut from the widest part of the ganglion 360 were counted.

As to size, one of the largest cells was 39 by 54 μ ; one of the smallest was 19 by 39 μ . The average size is about 30 by 39 μ , and there are regular gradations between the largest and the smallest.

The major diameters of nuclei were from 8 to 13 μ , and the minors from 8 to 11 μ . The processes in nearly all cases emerged without twists or turns and few implantation cones were observed.

In the mallard the ganglion of the ninth nerve is not so large nor is it bulged on one surface as in the common domestic duck. The arrangement of the cells in the ganglion is similar in both cases, except that in the mallard they are more evenly distributed about the periphery. The cells are smaller and more uniform in size than in the common duck. The largest measured 27 by 33 μ ; the smallest 16 by 19. The average of the cells is about 28 μ long and 23 wide. The cells were mostly elliptical in shape, some were rounded and many were irregular, but the irregularity is not due to the crowding of neighboring cells.

b. Vagus ganglion of the mallard. The vagus ganglion is quite small as compared with that of the common domestic duck. It

is a little over 1 mm. in width. The cells throughout the ganglion are arranged in single rows of four to thirteen separated by bundles of fibers. There is no crowding of cells about the periphery as was so commonly observed in the other ganglia. The predominating form of the cell in this ganglion is elongated. Many of them were very long and slender, one measured $82\ \mu$ in length and only $18\ \mu$ in breadth. It, like many others, is tapering toward both ends like a spindle. Of the remaining cells, the elliptical are more common, a few are pear-shaped, and the rest are rounded and irregular. The cell of largest volume was 30 by $63\ \mu$ and the smallest 14 by $27\ \mu$. There was a uniform gradation in the sizes from the largest to the smallest.

The nuclei are round to elliptical and vary in major diameter between 8 and $16\ \mu$ and in minor diameter between 7 and $13\ \mu$. But one nucleolus was observed in any nucleus.

The cells are surrounded by thin capsules in which are the characteristic nuclei. A rather limited space separates the cell from its capsule. The processes issue in nearly all cases in a direct line. A few bipolar cells were observed, one process being slender and the other considerably larger.

c. Gasserian ganglion of the domestic duck. The Gasserian ganglion of the domestic duck is very large. The one measured was 5 mm. long and over 2 mm. broad. The cells are slightly more numerous around the periphery than in the central region. Those in the body of the ganglion are separated into elongated groups with one to three rows in each group and 2 or 3 to many cells in each row. The larger number of cells are rounded, others are elliptical, pear-shaped, and irregular.

In major diameters the cells range between 22 and $74\ \mu$, and in minor between 19 and $44\ \mu$. The nuclei are larger than those observed in the cells of other ganglia of the duck. The largest measured 14 by $17\ \mu$, and the large ones were abundant. They ranged downward in size to one having a uniform diameter of $7\ \mu$. The pericellular capsule and its nuclei are like those in the other ganglion cells. The majority of the processes observed follow a direct course on emerging from the cell. A few had initial glomeruli, but no implantation cones were seen. A limited num-

ber of bipolar cells were observed having the characteristic large and small processes.

d. Gasserian ganglion of the mallard. This ganglion resembles in shape and in arrangement of cells the Gasserian ganglion of the common domestic duck, but the cells are much smaller and more uniform in size. One of the largest cells measured only 26 by 38 μ . Lobulated cells, varying from surface rugosities to distinct lobes, predominate in this ganglion. The nuclei are smaller and a larger percentage of them are rounded. Many of the cells are vacuolated, the vacuoles being usually small and situated near the periphery of the cell.

A large number of the processes formed arcs of greater or less degree about the cell, or followed a meandering course. Few initial glomeruli were observed, but a number of the processes issue from implantation cones.

E. SENSORY GANGLIA OF THE TURKEY

1. Cerebral ganglia

Observations on the sensory ganglia of the turkey were limited to those of the cerebral region.

a. Ganglion of the ninth nerve. In the turkey this ganglion is relatively small, its length being a little over 1 mm. and its breadth less than 0.5 mm. A careful count of a section through the widest part of the ganglion showed the presence of 134 cells. The cells are scattered throughout the ganglion without orderly arrangement.

The prevailing form of cell is elliptical, but rounded and irregular forms are not uncommon. The cells varied in major diameters between 26 and 52 μ , and in minor diameters between 15 and 33 μ . All processes observed emerged from the cells without twists or initial glomeruli.

b. The vagus ganglion. This ganglion is rather small in the turkey as compared with the same ganglion in the goose or duck. The cells are distributed, largely, in rows parallel to the long axis of the ganglion; they are more numerous at the distal end than in any other part of the ganglion.

The cells are mostly elliptical in shape, a number are rounded, and the remaining cells are irregular. The greater diameters vary between 14 and 55 μ and the lesser between 11 and 41 μ . The nuclei are round to elliptical, with the larger nuclei in the larger cells. The processes, where observed, issued without twist or initial glomerulus.

c. *The Gasserian ganglion.* The Gasserian ganglion of the turkey is also small in comparison with that of the duck and goose. It is about the same size as the Gasserian in the chick. The cells are much closer together than in the ganglia of the other large birds, and are arranged in groups having one to three rows of cells. The rows are usually of considerable length. The cells are nearly uniform in shape, being mostly elliptical with a few rounded and irregular forms. The major diameters range between 25 and 82 μ , and the minor between 14 and 50 μ . The nuclei are round to elliptical, the majority being rounded. No deviation from the usual way in which the processes issue from the cells of other birds was observed in the Gasserian of the turkey.

F. SENSORY GANGLIA OF THE PIGEON

1. *Spinal ganglia*

In the pigeon the ganglia of the brachial and lumbo-sacral regions are much larger than the other spinal ganglia, and those of the brachial region are considerably larger than those of the lumbo-sacral.

In the brachial ganglia the cells are somewhat more numerous about the periphery than in the central part. There is a decided tendency toward the arrangement of the cells into elongated groups consisting usually of single rows of cells. Here again, is seen the division of cells into two classes, large and small. The large cells are not numerous but are decidedly larger than the others. One of the average sized large cells was 35 by 44 μ . This cell had a rounded nucleus 14 μ in diameter. An average cell of the smaller size is about 30 μ long and 19 broad, with a nucleus of 7 by 11 μ . Most of the cells are elliptical, a few are rounded and irregular. The nuclei are relatively large and are round to elliptical in shape.

The processes, so far as observed, follow a direct course after they emerge from the cell. The delicate capsule and its elliptical nuclei are clearly shown.

The ganglia of the lumbo-sacral region have the cells arranged in rows. There is a less marked division of the cells into two classes, since many cells of intermediate size occur, forming transition stages between the large and the small cells. The spinal ganglia outside of the brachial and lumbo-sacral regions vary somewhat in size, although all are small. There is a slight tendency toward arrangement of the cells into elongated groups. The cells are distributed fairly uniformly throughout the ganglia.

The majority of the cells are rounded to elliptical, a number are pear-shaped, and the remainder are irregular. One of the largest cells measured $38\ \mu$ in length and 26 in breadth; while one of the smaller ones was $20\ \mu$ long and 12 broad. The nuclei are large, and in shape, round to elliptical, most of them being rounded. They are, as a rule, centrally located and in each was observed a large, clearly defined nucleolus.

2. Cerebral ganglia

a. Ganglion of the ninth nerve. In the pigeon this ganglion is very small, being less than 0.2 mm. in diameter, and a little less than 1.5 mm. long. The cells are distributed fairly uniformly throughout the ganglion. They are not close together nor is there any tendency towards arrangement in rows.

The cells are round to elliptical with here and there some that are oval, elongate, and irregular. The cells are relatively small, one of the largest observed being 16 by $27\ \mu$. One of the smallest cells measured $16\ \mu$ long and 11 broad.

The nuclei are relatively very large. One of the largest cells, 16 by $27\ \mu$, had a nucleus that was $14\ \mu$ long and 11 broad. A cell of average size, 16 by $19\ \mu$, had a rounded nucleus $11\ \mu$ in diameter. The nuclei were usually near the center of the cells, but not infrequently they were near the walls of the cells. The nuclei contain one very large nucleolus.

b. The vagus ganglion. In the pigeon this ganglion is less than 0.5 mm. in diameter and about 1.5 mm. long. The cells lie

much closer together at the proximal end of the ganglion than at the distal. There is but slight tendency for the cells to be arranged in rows.

The cells are smaller and more rounded at the proximal than at the distal end of the ganglion. At the distal end they are elongated and of relatively large size. A typical cell of average size, from the central end, had for its diameters, 22 and 16 μ , with a nucleus whose diameters were 11 and 8 μ respectively, while a typical cell from the distal end was 30 μ long and 16 wide with a nucleus 11 by 8 μ . The nuclei are relatively large, but not so large as those of the ganglion cells of the ninth nerve.

c. *Gasserian ganglion*. In the Gasserian ganglion of the pigeon the cells extend 0.8 mm. in a direction parallel to the course of the fibers, and 1.5 mm. in the direction at right angles to the course of the fibers. The cells are crowded very close together at the proximal end of the ganglion, and lack systematic arrangement, while in the rest of the ganglion the cells are less dense and are arranged in clusters consisting of one to four rows of cells, separated by bundles of fibers.

The prevailing form of cell is rounded; elliptical ones are much less numerous. Some are irregularly polyhedral on account of pressure of the surrounding cells. The cells are nearly uniform in size. They varied in length between 16 and 38 μ and in breadth between 14 and 27 μ . The larger and smaller cells are comparatively few. The nuclei are comparatively large but are proportionally smaller than in the ganglion of the tenth nerve.

G. SENSORY GANGLIA OF THE SPARROW.

1. *Spinal ganglia*

Observations were made only on the spinal ganglia of the sparrow. The ganglia of the lumbar region are quite small, one measuring about 0.5 mm. in length and about 0.33 mm. in breadth. The cells are much more numerous at the periphery than at the center of the ganglion. In the peripheral region there is no grouping of cells, while in the central part the cells are arranged in elongated groups of one to three rows.

The cells at the periphery are irregularly polyhedral in shape as a result of pressure from neighboring cells, while in the central region they are rounded to elliptical, the rounded forms predominating. The cells are relatively small. The largest measured had a uniform diameter of $27\ \mu$, with a rounded nucleus of $8\ \mu$. The smallest cell measured was round, with a diameter of $12\ \mu$ and a rounded nucleus of $6\ \mu$. The nucleus, as a rule, is situated near the center of the cell and is relatively quite large. A single darkly stained nucleolus was seen in each nucleus. The processes are fine and take a direct course as they emerge from the cells. No glomeruli or implantation cones were observed.

The spinal ganglia between the brachial and lumbar regions are very small, one of the larger of them measuring 0.5 mm. in length and 0.25 mm. in breadth. As shown in figure 40, the cells are crowded very close together, imparting to them irregular polyhedral forms. There is no grouping of cells such as is commonly seen in the ganglia of larger birds. The cells are smaller than those of the lumbar ganglia, the largest measured being 19 by $25\ \mu$. One of the smallest was triangular in shape and measured $11\ \mu$ from base to apex. The nuclei are nearly all rounded and are comparatively large. Most of the nuclei had a single deeply stained nucleolus, but in a few nuclei there were seen two nucleoli (fig. 40). A very delicate but clearly defined capsule (not shown in fig. 40) surrounds each cell.

IV. SUMMARY

The sensory ganglia of birds vary in size somewhat in proportion to the size of the bird. In the individual bird the order of size of the ganglia beginning with the largest is Gasserian, brachial, tenth, lumbo-sacral, the other spinals, and the ninth. The brachial ganglia are relatively larger in birds that use their wings much.

In larger ganglia the cells are more numerous about the periphery and in this position lack sympathetic arrangement, in the central region the cells are arranged in elongated groups. The smaller ganglia do not exhibit a definite grouping of cells.

The cells of the smaller ganglia of the small birds are quite crowded, while in the larger birds the cells of the smaller ganglia have considerably more room.

The size of the ganglion cells are in a measure proportional to the size of the bird. There is usually considerable variability of size in the cells of each ganglion.

In the brachial ganglia of both the owl and the mallard duck the cells are divisible on the basis of size into two classes, large and small, the larger cells being much less numerous.

The larger nuclei are usually found in the larger cells. In the small birds the nuclei are larger in proportion to the size of the cell than in the large birds.

The outline of ganglion cells of birds is usually rounded or elliptical with a small percentage of pear-shaped, club-shaped and irregular forms. The ganglion cells of old birds show more irregularities than those of young birds. Lobulated cells are common in the Gasserian and tenth ganglia of the owl and less common in the same ganglia of the mallard duck.

In adult birds the predominating type of ganglion cell is unipolar with branches of unequal size from the main axis—a larger branch going to the periphery and a small one to the central nervous system.

In embryos the ganglion cells are bipolar, beginning as oppositi-polar and exhibiting gradations up to the unipolar condition. In chick embryos of twelve to fourteen days incubation many intermediate forms are present.

Initial glomeruli and implantation cones are infrequent, except in the Gasserian and the tenth ganglia of the owl.

A remarkable coiling of the central axis of the peripheral process was observed in the Gasserian ganglion of the chick, the sheath not being affected.

The ganglion cells of the old hen are less plump than those of younger fowls and show many vacuoles. They also have a larger number of protoplasmic slings and fenestrations. Complicated fenestrations are present in the Gasserian ganglion of the old hen. Simple fenestrations were seen in the same ganglion of young birds.

Fine accessory processes, terminating within the capsule, some with and some without end enlargements, are present in the Gasserian ganglion of the old hen. Similar accessory processes were less frequently observed in the owl and other birds.

Pericellular networks were especially well shown in the vagus of the old hen. They occur in the ganglia of other birds, especially in the owl.

A close meshed perinuclear network was observed in ganglion cells of the Gasserian of the chick.

The cells of the sensory ganglia of birds are surrounded with connective tissue sheaths in which are elliptically-shaped nuclei. These nuclei are more numerous in the goose and duck than in the other birds examined. The ganglia also show mantle-like nuclei, in considerable numbers, in the intercellular spaces. No true multipolar cells were observed in sensory ganglia.

Non-medullated fibers from the sympathetic were observed in all ganglia studied and in some instances the fibers could be traced to pericellular networks.

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PLATES

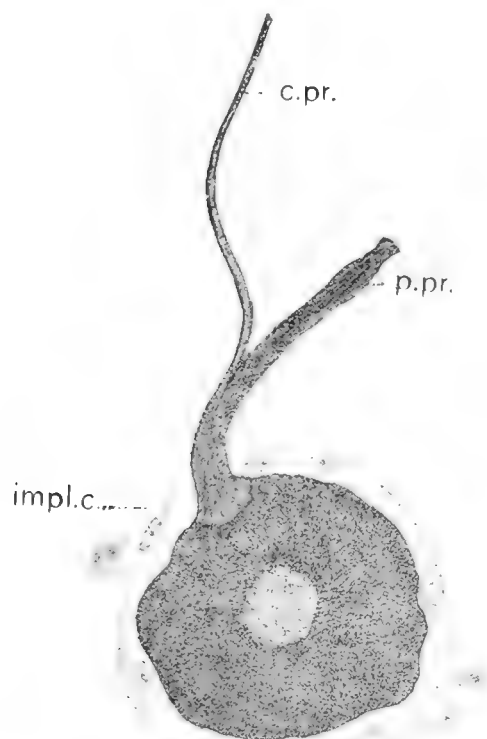
ABBREVIATIONS

<i>a</i> , individual cell referred to in the text	<i>Gass.</i> , Gasserian ganglion
<i>b</i> , individual cell referred to in the text	<i>glom.</i> , glomerulus
<i>b</i> ₁ , individual cell referred to in the text	<i>gnl.</i> , sensory ganglion
<i>b</i> ₂ , individual cell referred to in the text	<i>impl.c.</i> , implantation cone
<i>c</i> , individual cell referred to in the text	<i>L.</i> , large bundle in fig. 13
<i>ac.pr.</i> , accessory process	<i>lb.</i> , lobe
<i>af.pr.</i> , afferent process	<i>ncl.</i> , nucleolus
<i>c.pr.</i> , central process	<i>ntw.</i> , network
<i>cap.</i> , capsule	<i>nu.</i> , nucleus
<i>cap.nu.</i> , capsular nucleus	<i>p.pr.</i> , peripheral process
<i>et.c.</i> , nucleus in intercellular space	<i>pr.cl.</i> , pericellular network
<i>fbr.</i> , fiber	<i>slg.</i> , slings
<i>fen.</i> , fenestration	<i>tw.pr.</i> , twisted process
	<i>vac.</i> , vacuole

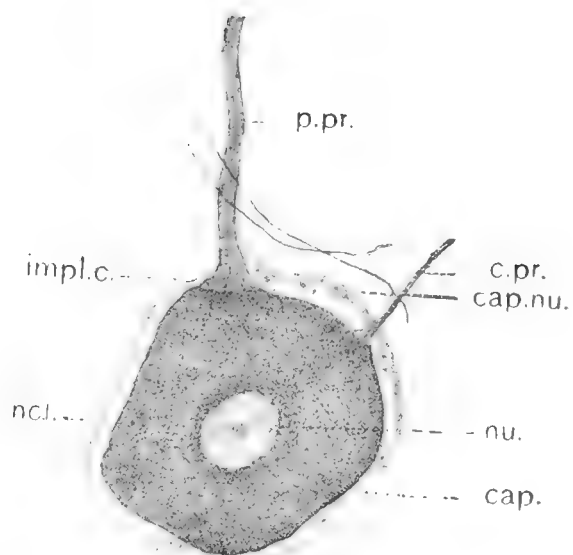
PLATE 1

EXPLANATION OF FIGURES

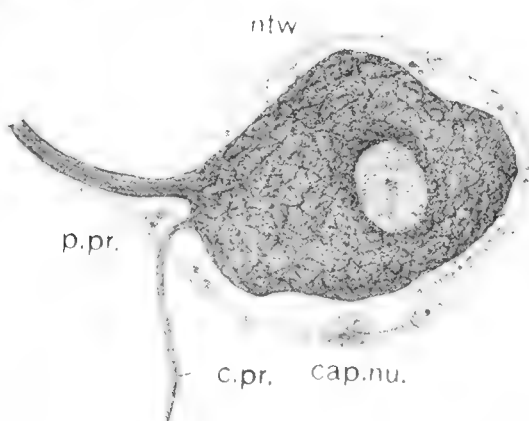
- 1 Typical unipolar cell from a spinal ganglion of a six-months-old chick. $\times 800$.
- 2, 3, 4, 5 Bipolar cells from spinal ganglia of the adult chick. Figure 5 shows the protoplasmic network of the cell body and of the processes. Note in the different cells the capsule, capsular nuclei, and the smaller size of the central process. $\times 800$.
- 6 Bipolar cell in which two processes have just come together. Observed in a spinal ganglion of an adult chick. $\times 800$.



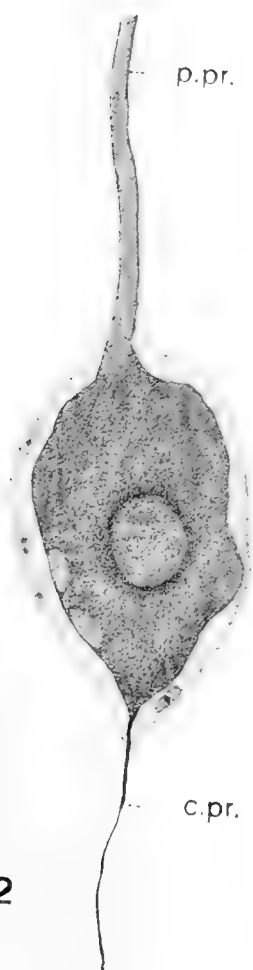
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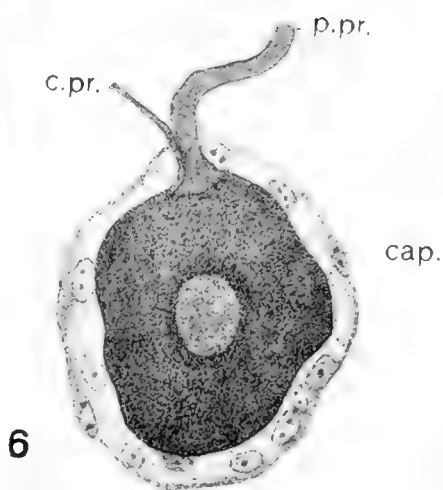
4



5



2



6



3

PLATE 2

EXPLANATION OF FIGURES

7 Cell from the spinal ganglion of the chick, the main process having an initial glomerulus. $\times 800$.

8 A spinal ganglion cell of the chick having fenestrations and slings near the periphery. $\times 800$.

9 Cell of the vagus ganglion of a six-year-old hen showing fenestrations, slings, and pericellular network. $\times 800$.

10 Cell from the same ganglion as figure 9, showing fibers surrounding the cell, and also part of the pericellular network of an adjacent cell. $\times 800$.

11 Much convoluted axis cylinder from the Gasserian ganglion of the chick, stained with silver nitrate and counterstained with Delafield's haematoxylin; the ends *a* and *b* should be continuous. $\times 2000$.

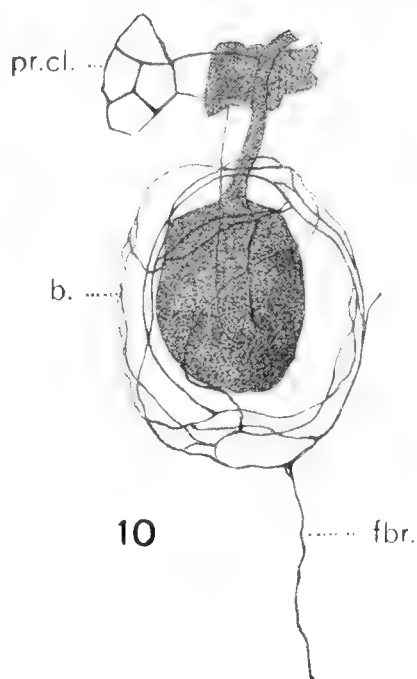
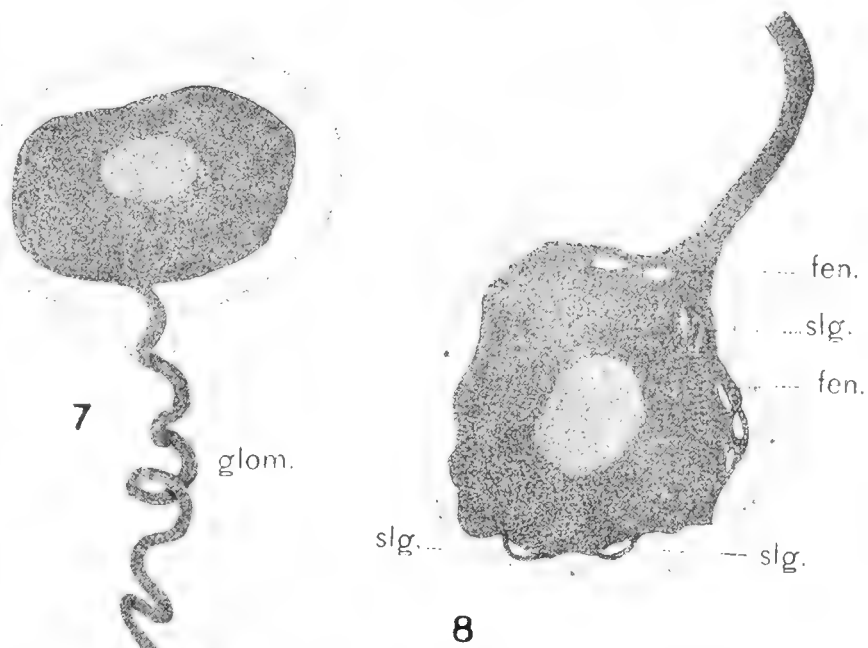
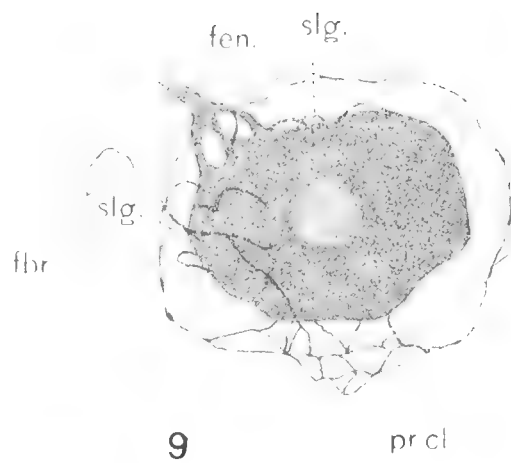
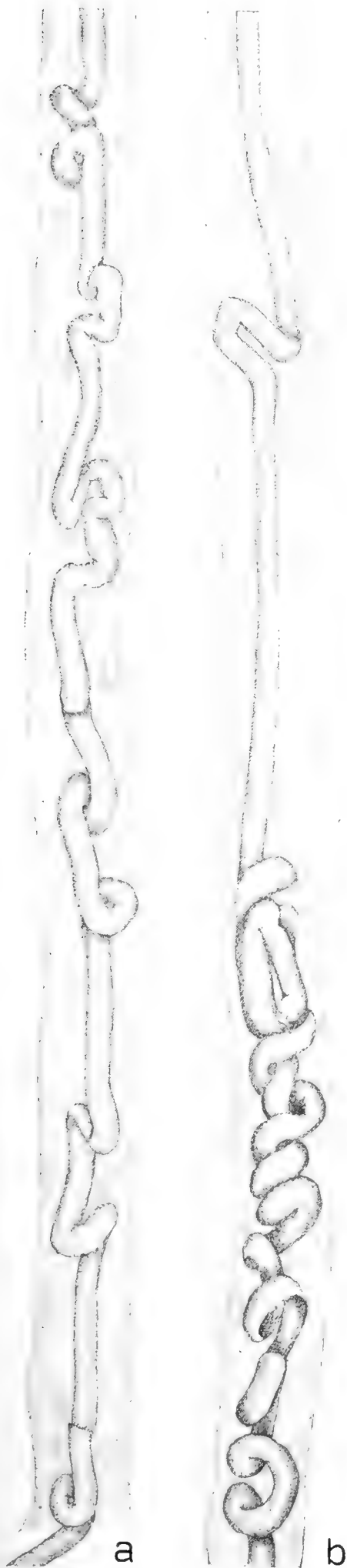


PLATE 3

EXPLANATION OF FIGURES

- 12 A section through the central part of the glossopharyngeal ganglion of the chick, indicating the relations of the elements.
- 13 A group of cells from the periphery of the vagus ganglion of the chick, showing typical cells and bundles of non-medullated fibers. $\times 1000$.

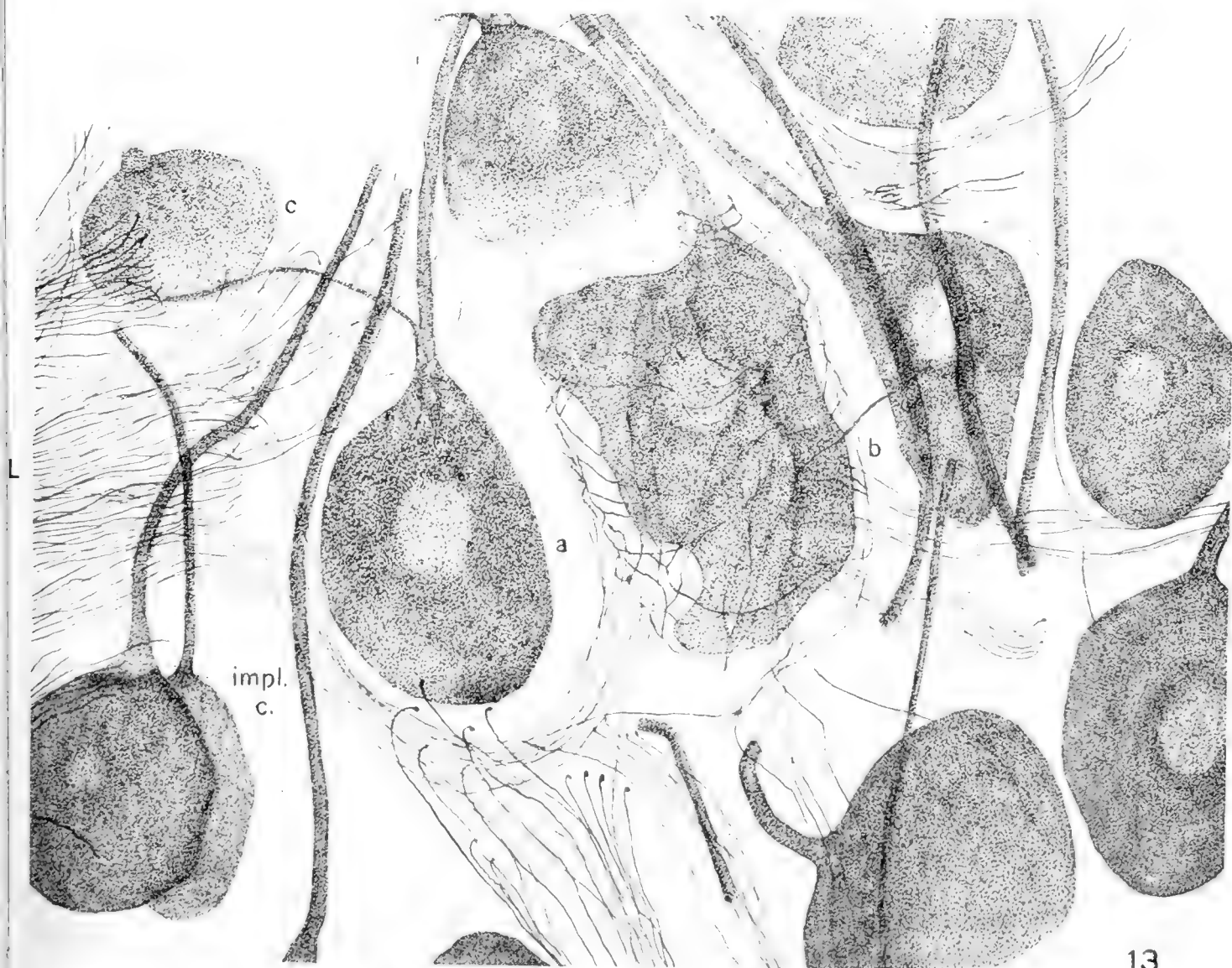
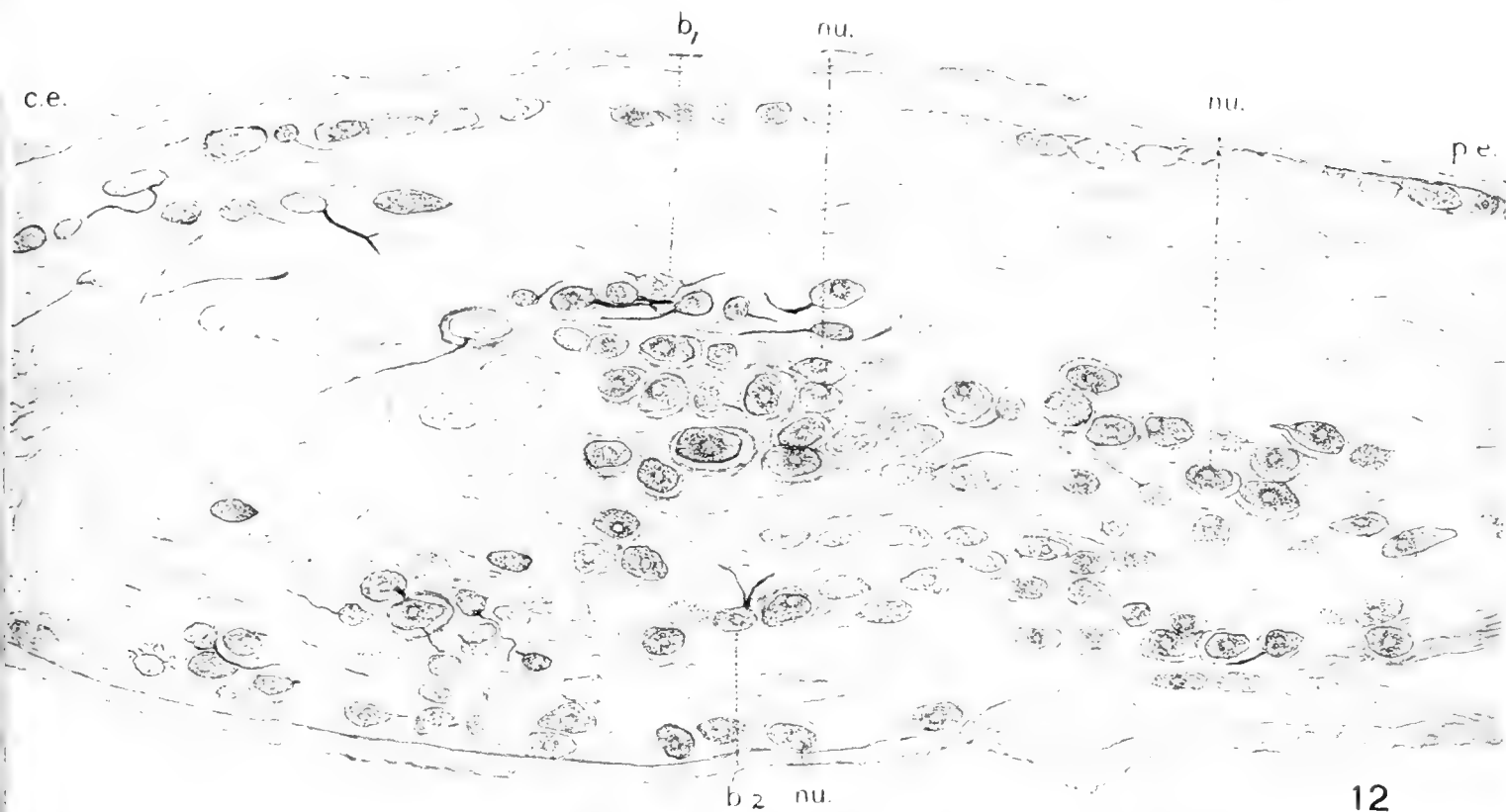
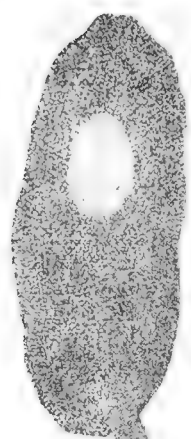


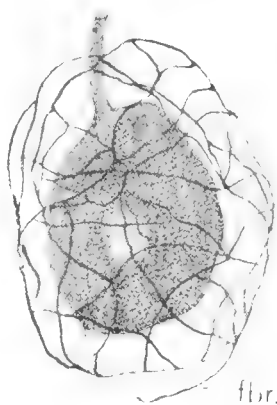
PLATE 4

EXPLANATION OF FIGURES

- 14 Cell from the vagus ganglion of a six-year-old hen showing pericellular network. $\times 800$.
- 15 Cell with initial glomerulus from the Gasserian ganglion of the chick. $\times 800$.
- 16 Cell with twisted process from the Gasserian ganglion of the chick. $\times 800$.
- 17 Vacuolated cell from the Gasserian ganglion of the old hen. $\times 800$.
- 18 Multipolar cell with accessory processes and protoplasmic slings, from a sympathetic ganglion of the chick. $\times 800$.
- 19 Cell showing accessory processes terminating in end bulbs, from the Gasserian of a six-year-old hen. $\times 800$.



14



fibr.

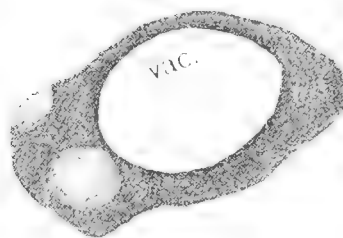


glom.

15

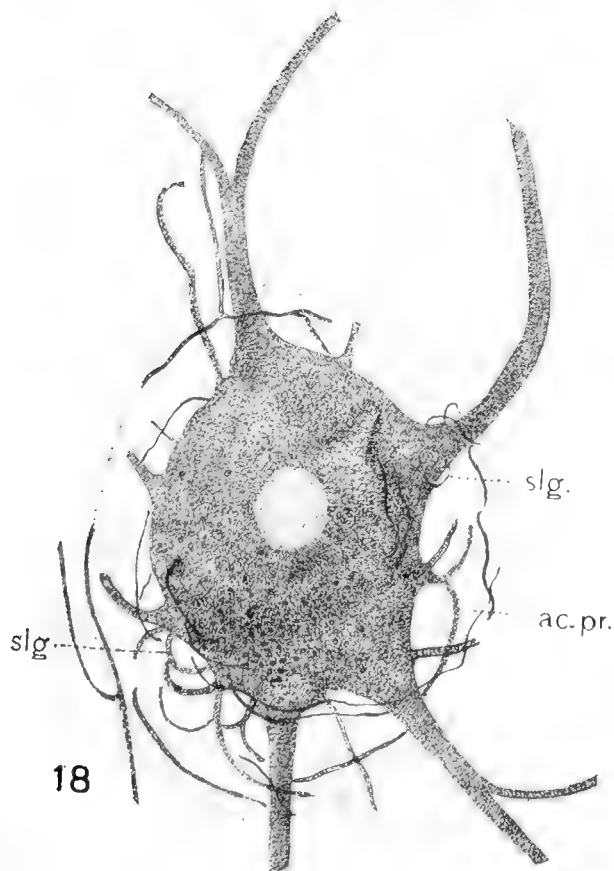
tw.pr.

16



vac.

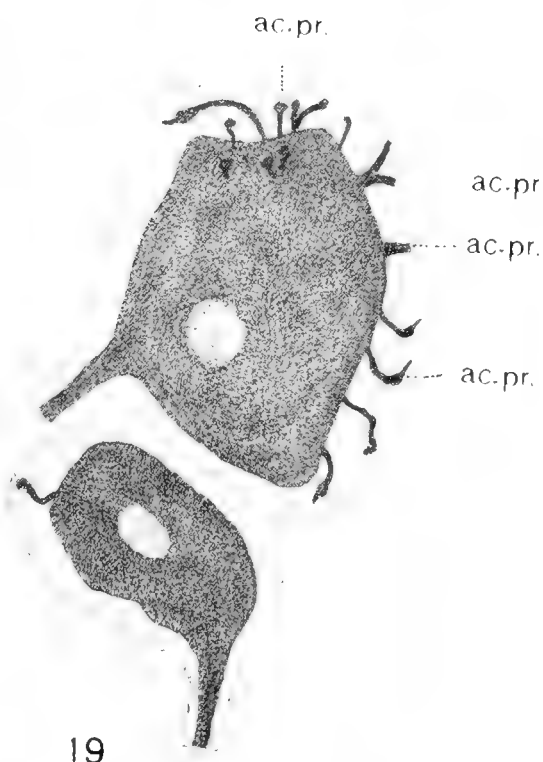
17



slg.

ac.pr.

18



ac.pr.

ac.pr.

ac.pr.

ac.pr.

19

PLATE 5

EXPLANATION OF FIGURES

20 Bipolar cell with fenestration and accessory process near the central process, from the Gasserian of a chick. $\times 800$.

21 Cell from the Gasserian ganglion of an old hen, showing slings about the axone and also at the opposite pole. $\times 800$.

22 Cell from the Gasserian ganglion of an old hen with slings and protoplasmic strands about the axone. $\times 800$.

23 Cell with a nest of protoplasmic loops on the pole opposite the main process, from the Gasserian ganglion of a six-year-old hen. $\times 800$.

24 Bipolar cell with protoplasmic loops and an accessory process near the central process, from the Gasserian ganglion of a six-months-old chick. $\times 800$.

E. VICTOR SMITH
fen. ac.pr.

c.pr.

20

p.pr.

slg

21

cap.nu.
nu.

slg.

af.pr.

p

a

p.pr.

23

nu.

ac pr.

c.pr.

slg.

24

ac.pr

22

PLATE 6

EXPLANATION OF FIGURES

- 25 Lobed cell with glomerulus and accessory processes, from the vagus ganglion of the owl. $\times 800$.
- 26 Irregular cell from the vagus ganglion of the owl. $\times 800$.
- 27 Cell from the vagus ganglion of the owl. $\times 800$.
- 28 and 29 Cells from the vagus ganglion of the owl showing fine accessory processes with end bulbs issuing from the main process. $\times 800$.
- 30 Cells from the vagus ganglion of the owl showing twisted process with fine accessory processes emerging from the main process. $\times 800$.
- 31 Lobulated cell from the Gasserian ganglion of the owl, with fenestrations and accessory processes. $\times 800$.
- 32 A lobed cell with a vacuole from the Gasserian ganglion of the owl. $\times 800$.
- 33 Cell from the same ganglion showing fibrillar network and initial glomerulus. $\times 800$.
- 34 Bipolar cells from the ganglion of the eighth nerve of the owl. $\times 800$.

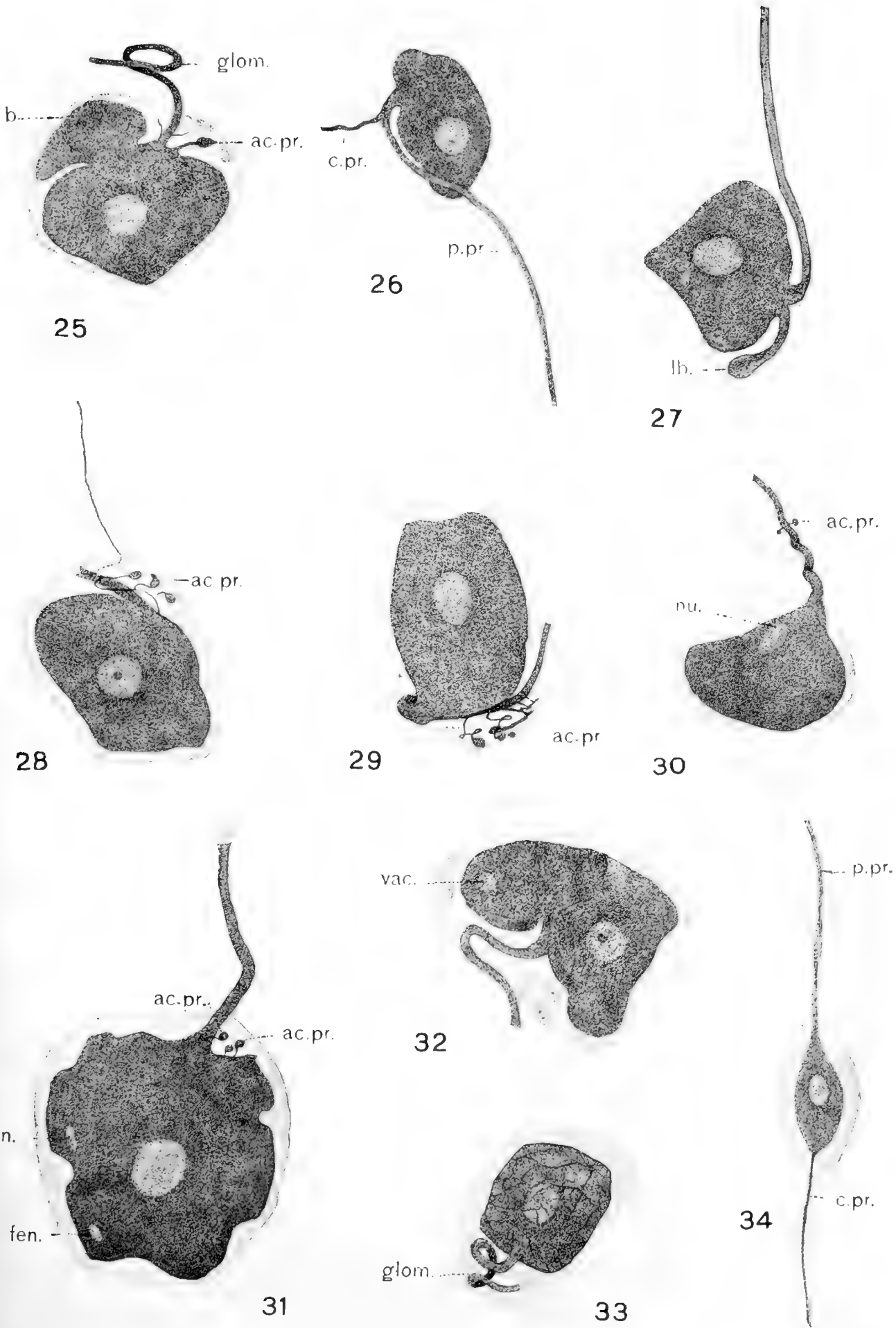
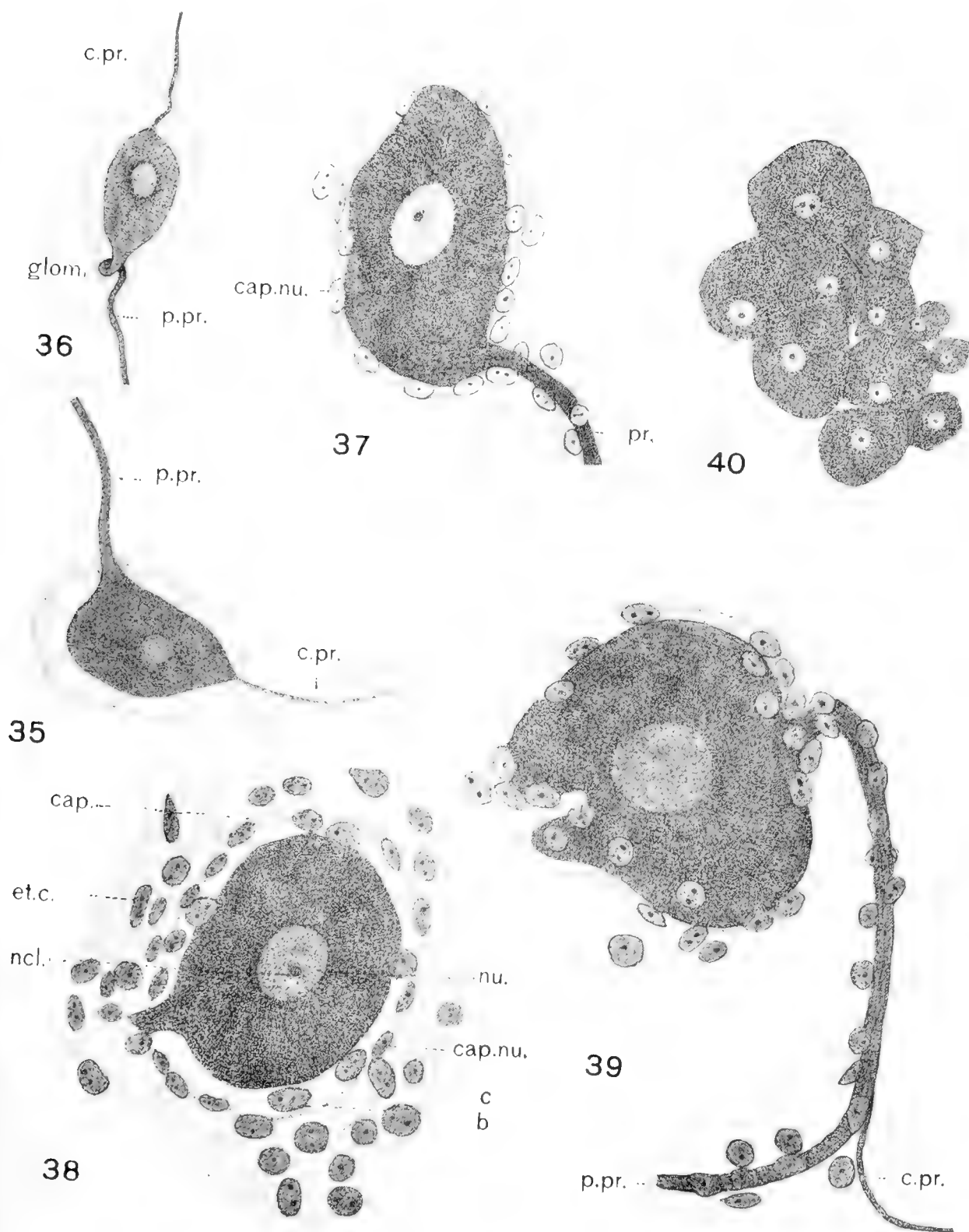


PLATE 7

EXPLANATION OF FIGURES

- 35 Bipolar cell from the ganglion of the eighth nerve of the owl showing a departure from the oppositi-polar condition. $\times 800$.
- 36 Cell from the auditory ganglion of the owl, showing glomerulus on the peripherally directed process. $\times 800$.
- 37 Typical cell from the glossopharyngeal ganglion of the goose. $\times 800$.
- 38 A typical cell from the Gasserian ganglion of the goose. $\times 800$.
- 39 A cell from the vagus ganglion of the goose, typical except as to the notch in the cell. Note its large size. $\times 800$.
- 40 A group of cells from a dorsal spinal ganglion of the sparrow.





FURTHER STUDIES OF THE HISTOLOGY OF THE THYMUS

ALWIN M. PAPPENHEIMER

From the Marine Biological Laboratory, Woods Hole, Mass., and the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York

TEN FIGURES (FIVE PLATES)

THE AMERICAN JOURNAL OF ANATOMY

ERRATA

The American Journal of Anatomy, Volume 13, Number 4, September, 1912

Page 428, description of figure 8, for (Reconstruction, fig. 32) read (Reconstruction, fig. 29).

Page 438, third line, description of figure 13, for figure 33 read figure 30.

Page 442, figure 16, for 4.5 read 4.S.

Page 472, description of figure 32, for figure 31 read figure 28.

Page 473, figure 33, for 5d and 5s, read 4d and 4s.

ABSENCE OF SUITABLE LYMPHOID TISSUE IN THE TROG, the observations were made on tissue obtained from young rats.

It may be admitted at the outset that clear-cut morphological evidence of a secretory function on the part of any of the thymic elements, has not been obtained. The observations which were made serve, however, to clarify some of the controversial points in the normal structure of the organ and add some new histological details which seem to justify their publication.

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TEN FIGURES (FIVE PLATES)

The conception of the thymus gland as an organ of internal secretion rests almost wholly upon the facts obtained from physiological experiment. In the thyroid, parathyroid, hypophysis and adrenal, we have clear morphological evidence of secretory activity on the part of the parenchymal cells. But this is not true in the case of the thymus, and we do not even know which of the complex elements of the gland contribute the hypothetical secretion. The following study of the frog's thymus was undertaken primarily in the hope of throwing some light upon this problem, by the use of methods which have not hitherto been applied to a study of the thymus; namely, stains for the demonstration of cell granulae, and the study of the living cells grown in vitro after the method elaborated by Harrison, Burrows and Carrel. The findings in the fixed tissue were checked up and amplified by applying various vital stains to the living cells in cultures. The work includes also a comparative study of the growth of thymus and lymph-node in vitro. Because of the absence of suitable lymphoid tissue in the frog, the observations were made on tissue obtained from young rats.

It may be admitted at the outset that clear-cut morphological evidence of a secretory function on the part of any of the thymic elements, has not been obtained. The observations which were made serve, however, to clarify some of the controversial points in the normal structure of the organ and add some new histological details which seem to justify their publication.

MATERIAL AND TECHNIC

The glands were obtained during the months of July, August and the early part of September from freshly caught specimens of *Rana clamata*; a few also from spotted frogs shipped from a distance and showing in consequence of the prolonged starvation, marked involutional changes. In addition to the usual histological methods a variety of granule stains were used: Altmann's acid fuchsin, as originally described and as modified by Lane; Bensley's neutral gentian violet (1); Heidenhain's iron-alum hematoxylin after fixation in Benda's modified Fleming's solution; and Benda's mitochondrial stain, used in the manner originally described by him and according to the modification suggested by Meves and Duesberg (2). The clearest pictures were obtained with the modified Benda method, although somewhat varying appearances were often presented by sections prepared with the same technic.

The preparation of in vitro cultures has been so often described that it is unnecessary to give the method again in detail. The plasma was obtained directly from the frog's heart by aspiration with an oiled needle or glass pipette. Hanging drop cultures were kept at room temperature, as it was found that all the elements rapidly degenerated when incubated at 37°. In studying the reaction of the cells to various vital stains, dilute sterile solutions of the dye in Ringer's fluid were added directly to the cultures. In this way, with the gradual diffusion of the dye through the plasma, the reaction of individual cells could be directly observed. In other cases, the vital stain was added to the plasma before implanting the tissue, but under these conditions, for reasons which will be discussed later, no growth was observed.

HISTOLOGICAL STUDY

In the adult frog, the thymus is an elliptical, slightly flattened yellowish body, from 2 to 4 mm. in length, situated on either side beneath the angle of the inferior maxillary, and in close relation to the glosso-pharyngeal nerve and the depressor man-

dibulae muscle. On the surface, one may see in the gross stellate masses of black pigment. The surface is smooth and shows no lobulation. After prolonged starvation, or in infected frogs, the gland shrinks, has a somewhat translucent appearance and may be difficult to recognize with the unaided eye. In old frogs, the texture of the gland becomes firmer and the color a deeper yellow.

Microscopically, one finds a sharp distinction between cortex and medulla, the relative proportions varying in different individuals. The cortex is composed principally of closely packed small thymus cells, but often shows a subdivision into an outer zone, in which the nuclei of the small cells are paler and show a more distinct chromatin network; and an inner zone, in which the nuclei are smaller, denser and more deeply stained. Mitoses in variable number are present in both strata of the cortex, but are probably more abundant in the periphery.

The medulla is formed in large part by the myoid cells, which in the frog's thymus are often a striking and conspicuous feature of the histological picture. These cells have in general an oval or circular outline which is quite sharply defined, although band-like forms occur. With Heidenhain's iron hematoxylin or with crystal violet after Benda fixation, they show a distinct fibrillar structure. The fibrils most commonly have a circular arrangement, and show an alignment of deeply staining rods or granulae, giving the appearance of distinct cross striation. In many cells of this type, however, there is no regular alignment of rods or granules, which may be quite irregularly disposed throughout the cell. Whether the granulae represent cross sections of short filaments, or true granulae, is often quite difficult to decide. Many cells show in their peripheral portion, regularly disposed striated fibrils, while in the central portion of the cell, these appear to be broken up into dots and lines. Cells of this type containing scattered clumps of deeply stained material, are interpreted as degeneration forms. When the granules or rods are at the same level in adjacent fibrils, the resemblance to the striations of muscle fibers is a close one, and the aptness of the term myoid is evident.

Very frequently, these cells contain one or more rounded cavities, and in some cases the cell is composed merely of a shell of striated fibrils, enclosing a single large central cavity. In one such cell observed in a living culture, the cell, which in its free condition had taken on a globular form appeared to be tunnelled by a cavity the rim of which was slightly more refractile and presented a distinct striation. The nucleus was apparently located in a lobular projection at one portion of the cell.

Two views are held as to the nature of the myoid cells,—one supported by Mayer (3), Pensa (4), Weissenberg (5), and others, that they are derived from muscle fibers which become included in the gland in the course of its development; the other supported by Hammar (6) and Pappenheimer (7), that they are modified elements derived from the reticulum. In sections, the appearances lend countenance to the latter view: fibrils may occasionally be traced from one cell to another and transitions between reticular cells and cells with poorly developed fibrils apparently occur. In teased preparations, these elements may be readily recognized by their large size, regular contours, refractile appearance, and by the presence of striations sometimes seen on careful focussing. They appear always under these circumstances, as sharply circumscribed, usually globular bodies, so that we must assume that their anatomical connection with the reticular syncytium, is extremely slight. Their capacity for migration and growth in plasma cultures, is also nil. They show no amoeboid activity, but retain their fixed form, accumulate fat and gradually degenerate, in marked contrast, as will be pointed out later, to the reticular elements. Evidence of contractility on the part of the fibrils was carefully looked for, but an abrupt change of form suggesting such a function, was not observed.

In sections, the reticular cells are differentiated from the small thymic cells principally by the character of their nuclei, which are larger, paler with a more distinct chromatin network. The outlines of these cells are indistinct, the cells forming a loose protoplasmic meshwork in the interstices of which lie the myoid cells and the small thymic elements. In the cortex, amongst

the closely packed small cells, one finds scattered nuclei of the large type, evidently belonging to the reticular cells, but the protoplasmic reticulum is obscured.

In the ordinary hematoxylin-eosin preparations, or in sections stained with polychrome methylene-blue eosin, there are regularly seen scattered cells with large, rounded eosinophilic granulations. These cells are found both in the cortex and the medulla, and are often but not always, in close relation with the connective tissue sheaths of the blood vessels. I have been unable to decide from my preparations whether these granulae always belong to leucocytes or myelocytes, or whether some of the reticular cells may not contain eosinophilic granules.

The foregoing brief description, which agrees in all essentials with that of previous workers on the amphibian thymus, but which ignores entirely the mooted points as to the histogenesis of the different cellular components, will however suffice for the present study. I wish now to describe the appearances found in sections fixed and stained according to Benda's method for the demonstration of mitochondria.

In successful slides, the nuclear chromatin does not retain the crystal violet, but is stained yellowish brown by the alizarine. When, for reasons which were not determined, the nuclei retain the violet stain, the cytoplasmic granules are much less readily demonstrable. These preparations, however, give excellent pictures of the mitotic figures, of the fibrils of the myoid cells, and bring out also certain coarser granules in the reticular cells, to which we shall refer later.

In good preparations, the nuclei, though unstained with the violet, are distinct. The chromatin of the small cells is in the form of clumps, from one to four in a nucleus, lying in an unstained clear space. Ragged threads extend from these to the nuclear membrane. The latter shows a tendency to retain the crystal violet, especially when differentiation is not carried too far. The nuclei are thus often bounded by a rather heavy purplish line. It is not possible to distinguish absolutely between the nuclei of the small cells and those of the larger reticular elements. In general, the latter show a more delicate and evenly

distributed chromatin network, which is not in the form of large discrete clumps. The cell limits cannot be made out clearly, even in very thin sections (2μ). Occasionally in the cortical portion, the small thymic cells show a distinct outline, the nucleus being bounded by a narrow rim of brownish protoplasm, which is thicker at one pole of the cell, where the nucleus commonly shows a slight dell or indentation. In the medulla, where the different types of cells are intimately commingled, the nuclei appear separated by an indefinite protoplasmic substance. It is thus exceedingly difficult to determine to which cell the cytoplasmic granulae belong. Scattered between the nuclei, but never in or upon them, are very numerous minute purple granulae (fig. 1). These vary somewhat in size, but are in general smaller than the smallest coccus, and in some cells are barely within the range of visibility. (Comp. OC/6, Imm. 1/12 in.) The abundance of the granulae depends largely upon the degree of differentiation in acetic acid and alcohol. In sections which have been well differentiated, the granulae are fewer in number, but are more distinct, standing out sharply from the pale brownish background.

Instructive pictures are found near the ragged edge of sections in areas in which the small thymic cells have been dislodged or fallen out, and only the protoplasmic reticulum persists. Here the protoplasmic meshwork is found studded with innumerable, minute sharply defined granulae.

Whether the small thymic cells also contain granulae, or whether these are limited to the reticular cells, is difficult to decide from a study of the sections alone. The granulae are present in the cortical portion of the gland, in some sections in considerable abundance. Often they are in close relation to the nuclei of the small thymic cells, and in favorable cases, where the cells have become separated by a break in the section, they appear to lie within the narrow zone of protoplasm on one side of the nucleus. Often they form a row lying between adjacent cells, and they adhere to, or are incorporated with individual cells which have become loosened from their surroundings. From a study of the sections, the impression was gained that the small cells as well as the large reticular epithelial elements, contain

granules (fig. 2), and this was confirmed by a study of the living cells by means of vital stains.

While the protoplasm of the reticular cells has a fine fibrillary structure, it was not possible to demonstrate fila to which the granulae bore a definite arrangement. An alignment of the granules in rows was not found in the reticular cells, in the sections. In the myoid cells, however, the granulae or rods are arranged in definite rows at regular intervals upon a fibrillar groundwork, and to this is due the apparent cross-striation of the fibrillae and their resemblance to muscle fibers.

While the granules and filaments of the myoid cells are readily demonstrable by all the mitochondrial stains, they appear with equal distinctness after formalin fixation and prolonged staining with Heidenhain's iron hematoxylin after mordanting in 4 per cent alum. They are thus chemically distinct from the granulae of the reticular and small thymic cells which cannot be brought to view by this simple method.

In sections stained according to the Meves-Deusberg modification of the Benda method, the granulae are less numerous but more distinct. They occur in groups in certain of the reticular cells, and are quite variable in size, the largest being about half as large as the nucleus of a small cell; the largest granules or droplets may show partial decolorization. They are almost always spherical, but occasionally are slightly elongated and somewhat irregular (figs. 3 and 4).

There remain to be noted certain cells with coarse slightly rod-shaped or bacillus-like granules, which stain intensely with crystal violet, even after fixation in Zenker-formol. These cells are scattered irregularly through cortex and medulla. They are not numerous but by reason of their deep staining and the large size of their granules, are very conspicuous. These cells are probably identical with the gentianophilic cells described by Prenant (8). Their identity with the eosinophile cells is questionable, although Prenant suggests this possibility, and states that the eosinophiles of the blood are also gentianophilic. The elongated character of the granules would, however, serve to differentiate these cells from the eosinophiles in which the granules are spherical.

OBSERVATIONS UPON LIVING CELLS

In the study of the living cells in vitro, certain difficulties were encountered, and the chief of these was the uncertainty in regard to the derivation of the growing elements. It was only after the study and comparison of a large number of different preparations that certain types of cells could be recognized, classified and their origin made reasonably certain. As has been observed by all workers, the growing cells do not adhere to their differentiated form, but assume a simpler and often indifferent type. The recent work of Foot (9) upon the growth of bone-marrow in vitro, emphasizes the difficulty in identifying the growing cells with definite constituents of the normal tissue.

Even under approximately identical conditions, so far as they can be analyzed, there is considerable variation in the extent of the growth, and to a less degree, in its character. Thus of a given series, only a certain proportion yields a definite growth, others gradually degenerating. While such discrepancies are undoubtedly due in large part to technical faults, it is rarely possible to find the exact cause for the failure of growth.

That the age of the frog from which the thymus is obtained has some influence upon the growth, is suggested by the following series. Two parallel sets of cultures were made, from half of which the thymus was taken from a young frog (6 cm.), and for the others, from a very large frog (9 cm.). The plasma of the small frog was used for both series. Eighteen preparations were made. Abundant growth was obtained in all but two of the cultures of 'young' thymus, whereas only three of the cultures of 'old' thymus grew and these but sparingly (33 per cent). In another series, eight preparations of thymus from an old very large frog, failed to show growth, while three controls of young frog thymus in the same plasma, showed abundant growth. There was unfortunately no opportunity to repeat these observations on a large scale, but the point deserves further study because of its bearing upon the involution of the thymus in adult life. It might be supposed that the plasma of old animals would exert a deleterious influence upon the growth of the thymic elements, but this has proven not to be the case. Some of the

best growths obtained took place in the plasma of very large, and presumably old frogs.

The influence of mechanical factors has been repeatedly emphasized by workers with this method. The direction of the growth, as well as the configuration of individual cells, is determined in large part by the direction of the fibrin threads which act as support.

In a successful preparation, one may observe the following sequence of events. In the teasing of the fragment, many small thymic cells are often separated from the main fragment, and are then distributed far into the surrounding plasma. The central bit of tissue soon becomes surrounded by a fringe or halo of isolated cells. Most of these are cells of the lymphoid type, but one may identify coarsely granular cells (eosinophiles?) and scattered myoid cells, the appearance of which in the living has already been described. After a few hours, the isolated small cells sink to the bottom of the plasma. Some however, remain adherent to the cover-glass and migrate out to a considerable distance from the main fragment. As regards the character of the amoeboid activity of the small cells, it is, as Hammar (10) has pointed out, identical with that of the small lymphocytes of the blood, as described by Jolly (11), Askanazy (12), Meves (13) and others. Lobe-like hyaline pseudopodia are extruded and retracted, first from one portion of the protoplasmic margin, and then from another. Often these pseudopodia are long and hair-like, and a number of such delicate filamentous processes, which may reach a length several times the diameter of the thymus cell, project from different points of the circumference. When there are active movements of progression, the pseudopodia of this type seem to be dragged astern like a rudder. The entire cell may become constricted into two lobes and appear to be about to divide, the nucleus changing its contour somewhat with the changing contour of the plasmatic prolongations.

The amoeboid activity of certain of the small cells may be maintained for six days or more. Especially at the periphery of the fringe of isolated cells at the bottom of the plasma, one finds many active cells. If a single cell be observed for a pro-

longed time, one may sometimes note alternating phases of activity and rest; during the latter phase, the cell assumes a spherical shape. Multiplication of the cells does not take place in vitro to an appreciable extent. On but two occasions was actual division observed. One of the cells in a twenty-four-hour culture, was first seen at 8.50 A.M. in a state of active amoeboid motion. Sketches were made at one minute intervals. The cell continued active until 9.47, crawling along the surface of an adjacent large cell. At this time it went into a resting spherical state, remaining inactive until 10.34 A.M. It then again became active, throwing out long pseudopodia and changing its relative position. At 10.45 it again became rounded and motionless. At 10.50 a shallow constriction was first seen dividing the cell into two equal lobes. This rapidly deepened, and at 10.51, the separation was complete, division having taken place in less than two minutes from the first appearance of the constriction. The two cells remained close together until 11.20 when the observation was interrupted. At 1.30 P.M. they had moved apart and could no longer be identified.

The second cell came under observation first at 2.29 P.M. Until 3.12 it continued to show active amoeboid motion; at this time it became quiescent, remaining so until 4.01, when a slight constriction was first noted. Division was complete at 4.05 and ten minutes later, one of the new cells became active and moved away from the other. During the division, a slight indication of a spindle was noted, but the chromosomes could not be definitely made out, and the mitotic character of the division could not be established with certainty. In none of the fixed and stained preparations could karyokinetic figures be found, although pictures suggesting amitosis were plentiful. The proliferative capacity of the lymphoid cells in vitro is therefore a limited one, and multiplication occurs to a negligible extent. The cells retain their vitality, however, for a considerable period, and amoeboid activity has been noted after six days and probably persists even longer. The majority of these cells are of small size and remain indefinitely in an inactive spherical shape. They seem to become denser and more refractile.

The degenerative changes which occur in the small thymus cells are of two types. There may take place a simple lysis, in which the cells become paler, losing their refractivity and finally appearing as mere shadows. In fixed preparations, the nucleus no longer stains and only a faint outline remains. More commonly the nucleus becomes smaller and more refractile and stains intensely and diffusely with the nuclear dyes. Occasionally, smaller particles are constricted off from the nucleus and extruded from the cells, being found free in the plasma. Whether the reduction in the size of the nucleus is accomplished wholly in this way, is not certain. In fixed preparations of older cultures, the majority of the small cells take on this form. The bizarre radial extrusions, which are seen in the mammalian thymus under circumstances leading to acute involutional changes, were not often found in the cultures. This is rather surprising, since the small cells of the thymus of infected or starving frogs show these changes in marked degree.

Under certain circumstances which were not accurately determined, there takes place an accumulation of fat in the small cells. The most frequent appearance is the presence of three or four rather large fat droplets in the cap of protoplasm corresponding to the dell in the nucleus; but scattered droplets may be found anywhere in the rim of protoplasm surrounding the nucleus. In some preparations, almost every cell contained a single larger droplet.

No microchemical study as to the nature of this lipid material was made. The droplets are highly refractile and stain brilliantly with Scharlach R. According to the studies of Holmström (14) and Hart (15) on the rabbit and human thymus, fat droplets, or lipid granules demonstrable by Ciaccio's method are normally absent from the small thymic cells; and Ciaccio (16) has found that the lymphoid cells of the blood contain no recognizable fat or lipid substance. Stheeman (17) makes a similar statement in regard to the lymphoid cells of the lymph-nodes. From the above observation, however, one is forced to conclude that the small cells of the amphibian thymus may, under certain conditions, accumulate fat in visible form. Since the fat drops

may be present in cells which show active amoeboid motion, and appear otherwise healthy, there is no reason for considering the change a degenerative one, but rather, in accordance with modern ideas, as an evidence of sub-oxidation.

In the description of the fixed material, it was stated that granulae could be demonstrated with reasonable certainty in the small thymus cells. The following 'vital' stains, applied by adding dilute solutions in Ringer's fluid to the cultures, showed the presence of granulae; neutral red, trypan-blau, and Janus green. With trypan-roth, isamin-blau, and new methylene blue GG, the refractivity of the protoplasm is so changed that the minutest fat droplets appear with great distinctness, but there is no actual staining of the granules. By adding neutral red to the culture, some but not all of the cells will be found to contain a few granules. It was not however, possible to demonstrate granulae in the small cells by injecting strong solutions into the dorsal lymph-sac, and after general diffusion of the dye had taken place, teasing the thymus in Ringer's solution. Red stained granules and larger masses of colored material are found in other cells, particularly the myoid cells by this method, as well as when added directly to the plasma.

With trypan blau, fine discrete granulae are found in many of the small cells. These are sharply localized to one segment of the cell, forming a dark bluish disc or cap which is very striking when seen with the low power. After a time, the nucleus gradually takes a faint bluish tinge.

With Janus green, extremely distinct granulae appear in some but not all of the cells of the small type. The dark greenish color of the granulae develops slowly, reaching its maximum intensity after about ten minutes. They are seen as sharply circumscribed dots, which vary from barely visible points up to the size of a large coccus. They are grouped at one pole of the cell facing the indentation of the nucleus and show no recognizable radial or linear alignment. They appear to be more numerous and attain a larger size than the granulae seen in Benda preparations, but they correspond closely to these as regards their location in the thicker portion of protoplasm facing the depression of the nucleus.

If a very weak solution of the dye be used, the nucleus remains unstained, but with a stronger concentration takes a distinct purplish-red tinge. It is generally held that nuclear staining takes place in dead cells (Plato (18), Fischel (19), Cesaris-Demel (20)). Since we have no absolute morphological criteria for distinguishing a living from a dead cell, it is probably more accurate to say that staining of the nucleus takes place only in a cell which is dead or injured. The converse is not true, however, and all dead or injured cells do not show nuclear staining with the vital stains.¹

Now it is certainly possible by means of Janus green to bring about a simultaneous contrast staining of granulae and nucleus in living cells. While I have not observed amoeboid activity in the small cells, nor any other functional indication of life, in the case of other elements which will be described in detail, marked active changes of form took place after staining was completed. The appearance of a reddish color denotes a reduction of the dye-stuff, or in other words a taking up of oxygen on the part of the nucleus, and is in all probability in itself an indication of viability on the part of the nucleus. The recent experiments of Warburg and Meyerhof (24) which show that crushed fragments of sea-urchin eggs and even acetone extract powders are capable of taking up oxygen for several hours, perhaps weakens the force of this argument. More convincing is the fact that dead nuclear material which is taken up by phagocytic cells, stains greenish blue and not reddish.

¹ To this general rule that staining of the nucleus indicates death or injury to the cell, there are a few exceptions noted in the literature. Thus Przemicki (21), working with certain Protozoa (*Opalina*, *Nyctotherus*) succeeded in staining the nucleus in individuals the motility of which was preserved for five days and in which cell division occurred. Goldman (22) in a recent paper states that he has succeeded in vitally staining the nuclei of liver cells. Kite and Chambers (23) announce in a preliminary note that they have produced a differential staining with Janus green of the chromosomes in the spermatic cells of the squash-bug, crickets and grasshoppers, and they have followed the transformation of anaphase to telophase in a stained spermatocyte. In some observations made by the writer in collaboration with Dr. R. A. Lambert, upon dividing cells of chick embryos in vitro, it was found that the chromosomes were stained red by Janus green, but that division of the cells was arrested upon the addition of the dye.

So far, only the changes occurring in cells of the small type have been considered. But there takes place also a definite outgrowth of cells which differ widely from the lymphoid elements and cannot be confused with them. The first evidence of growth is the projection of delicate protoplasmic sprouts from the margin of the main fragment. These have been seen as early as six hours after the preparation of the culture, but they may not appear until the following day, and rarely the first sign of growth is not seen until the second day. Following the appearance of the sprouts, large cells wander out into the medium, either as isolated cells or as coherent tissue-like planes of compact cells. Often these cells take on an elongated spindle shape and arrange themselves in long rows joined end to end, following a fibrin thread or the line of retraction of the plasma. When the cells grow out along the cover-glass, they become flattened, irregularly pyramidal or oval in shape, with long dendritic, barely visible plasmatic processes uniting them to the central fragment or to each other. The individual cells, as seen by the figure (fig. 7) are often of extremely large size, but are so irregular in shape that it is difficult to give measurements of value. The nucleus is relatively large, elliptical, though sometimes indented by large fat droplets in the cytoplasm. It is usually possible to distinguish one or two slightly more refractile nucleoli; otherwise, the nuclear substance appears homogeneous.

From their first appearance these cells are found to be filled with numerous granulae. At first, these are of small and uniform size, and but moderately refractile; after several days of incubation, the cells contain in addition, many droplets of varying size, which are refractile and evidently fat droplets. The smaller droplets or granulae often range themselves in rows of considerable length. The cell processes are relatively free from granulae, but occasionally do contain small granules, or larger fat droplets. One frequently sees knob-like thickenings along the course of a long plasmatic process in which such granules are found. Where the prolongation of the cell ends blindly, the termination is frayed into delicate hair-like processes, which because of their slight refractivity, appear to shade off into the plasma.

Often a fibrillar structure of the cytoplasm is recognizable in the living cell, the fibrils being especially distinct in the long slender protoplasmic processes. The appearance of these cells and their usual manner of growth, is indicated in figures 5, 6 and 7.

Changes of contour and relative position are readily detected in these cells, if the observation be sufficiently prolonged. They do not, however, under normal conditions, show as rapid changes of shape, as do the cells of the lymphoid type. After the first days of active emigration and growth, they retain their position and outline unchanged for many days, gradually accumulating fat drops in their protoplasm.

An interesting phenomenon which was seen in the living cells, and confirmed by subsequent fixation and staining, is the separation of portions of protoplasm, which gradually become constricted off and are set free into the plasma. The separated portions contain fat drops and granulae. The process resembles curiously the formation of blood platelets from megakaryocytes, as first described by H. Wright (25). Its significance here is uncertain; it may be found in cells which show no other degenerative changes.

The stained preparations of these cultures give somewhat varying pictures according to the method of fixation. After formalin, followed by iron-hematoxylin (Heidenhain), the nuclear substance stains homogeneously, the intensity depending upon the extent of decolorization. There are one or two nucleoli of large size, deeply stained, but often with a slightly paler center. There are in some of the nuclei, minute granules surrounded by a clear halo. The cytoplasm shows a beautifully reticulated structure, being composed apparently of delicate fibrillae, occasionally parallel in their course, but disarranged by the presence of smaller and larger fat vacuoles. Along the meshes of the reticulum are deeply staining granulae of smaller and larger size. Most of them are larger than the granules of the eosinophile cells. They are usually spherical, but may be slightly elongated, or when in apposition to a large fat globule, crescentic. The numbers vary;

in some cells closely aggregated, in others less numerous. They may extend out into the cell processes; usually there is a narrow zone at the surface of the cell which contains fewer granulae. They are often grouped in linear alignment about a fat vacuole, or in the long axis of the cell and especially in the protoplasmic processes where the protoplasmic fibrils run parallel. The granulae do not always stain with the same intensity in the same cell, but this may be due to uneven penetration of the stain or decolorizing agent through the plasma (figs. 5 and 6).

In most of the cells there is an area adjacent to the nucleus where the protoplasm is denser and the granulae and fat drops are absent. Although centrosome or cytasters were not seen in these preparations, it is probable that this denser granule-free area represents the cytocentrum.

When the cultures are fixed in bichloride-acetic acid, the cytoplasmic granulae are very indistinctly brought out by the Heidenhain stain. The nucleus on the other hand, instead of appearing homogeneous as in the formalin preparations, shows finely distributed chromatin clumps. The only mitotic figures seen in these cells during the course of the work, were in a specimen fixed in bichloride-acetic acid. The chromatin threads of the dividing cells (prophase and diaster stage) were very large and distinct.

The cytoplasmic granules of the cells were also demonstrated by the Altmann-Bensley method, the loosening and retraction of the clot being prevented by preliminary fixation in 10 per cent formalin. The stained preparations however were unsatisfactory since the decolorization of the fuchsin in the plasma clot was impossible, and only a few cells which by fortunate chance, lay within the retraction zone, were available for study.

By this method, smaller rounded cells filled with fuchsinophile granules were also seen. The granulae in part show swelling and disintegration, but may persist in this altered form even after the cell has degenerated.

The granulae of the large cells may be brought out clearly by the use of vital stains, the most successful of those tried being Janus green and methylene-blue GG. With the former stain,

the granulae gradually assume a dark greenish color, reaching its maximum intensity after eight or ten minutes. The concentration of the dye appears to have slight influence upon the rate of staining, but as in the case of the small cells, concentrated solutions produce a reddish staining of the nucleus. The smallest granulae appear to stain first and most intensely, and transitions of every degree are noted up to the large refractile unstained fat drops. The largest droplets often have a pronounced greenish tinge in certain focal planes; whether this is due to refraction of the color from surrounding stained granules, or whether the fat globules are enclosed in a stained shell, could not be decided. Without entering into a discussion of the rôle of the cell granulae in the synthesis of fat, it may be said that the appearance noted rather suggests the direct transformation of the granules or a portion of them into fat. Not only are there apparent transitions between the stained granules and the larger refractile droplets, but in Sudan preparations, the fat droplets may be of extremely small size, and distributed in the same linear alignment as the normal granulae. An attempt was made to examine more closely into the relation of the granules to the fat drops, by fixing the vitally stained cell, and subsequently staining with Sudan III; but it was found that the Janus green was rapidly decolorized by the formalin.

As the staining with Janus green progresses, there occurs a remarkable contraction of the entire cell. The long plasmatic prolongations are shortened, thickened and gradually withdrawn into the cell body. The entire cell becomes plumper and tends to assume a globular shape, the granulae and fat drops becoming clumped about the nucleus. The phenomenon may occupy only a few minutes. After having taken on a spherical shape, the cell for a time extrudes rounded ectoplasmic pseudopodia in various directions. This takes place even when the nucleus is distinctly stained reddish by the dye, and as has been said, this affords an example of nuclear staining in a cell which though injured, still shows vital activity.

Gradually these amoeboid extrusions cease, and the cells remain indefinitely in a globular form. The staining, however, fades

completely within twenty-four hours, the cell retaining only a faint diffuse greenish tinge.

If the Janus green be added originally to the plasma in which the tissue is implanted, no growth or emigration of cells occurs. This is probably due to a toxic effect of the stain as well as to an inhibition of amoeboid activity.²

With new methylene blue GG, in very dilute solutions, there is also produced a very sharp blue staining of the cytoplasmic granules. The nucleus remains unstained, and retraction of the cytoplasmic processes does not occur to any extent. The small thymic cells take a pale diffuse bluish tinge, but the granules though made visible by the altered refractivity, do not themselves stain.

Before discussing the origin of these large growing cells, mention should be made of their power to phagocyte the small thymic cells. Both in living and fixed preparations, the presence within the large cells of more or less intact small cells is easily recognized. Often the nucleus of the ingested small cell stains intensely with the vital stains, such as the new methylene blue, when the majority of the extra-cellular small cells are unstained. It may be fairly assumed from this that the ingested cell is dead. Such staining of the phagocytosed cell does not always occur, and the same phagocyte may contain both stained and unstained nuclei of the small cell type. Morphologically, the ingested cell may show no degenerative change save a condensation and increased refractivity of the nuclear material.

Stainable granulae may be found in considerable numbers in these phagocytic cells, distributed between the ingested small cells, and fat droplets. This observation is not in accord with the statement of Schulemann (26), that cells which have used up their 'receptors' in the process of phagocytosis no longer give

² Through the kindness of Professor Wherry, who supplied me with a culture, it was possible to try the effect of the dilute solutions of Janus green upon the motility of *Amoeba limax*. Amoeboid activity was completely inhibited after a few minutes, the amoebae taking on a globular form. A control on the same slide, to which a drop of Ringer's solution was added, remained actively motile after twenty-four hours.

a vital staining of the granules with trypan-blau. He found that macrophages from lymph nodes, when filled with erythrocytes, no longer contained stainable granulae. In the cells under consideration, the phagocytosis of other cellular elements is not accompanied by a disappearance of the granules.

There occurs then, in plasma cultures of frog thymus, a growth and to a limited extent, a multiplication of large cells of varying morphology, but evidently identical origin. The growth may be in the form of fairly compact, tissue-like sheets of cells, in a loose anastomosing reticulum, in long chains of cells joined end to end, or the cells may be entirely isolated, rounded or with plasmatic prolongations of varying tenuity and length. All types of growth and transitions between them may be seen in one and the same culture. The character of the nucleus in all the cells is essentially the same, but its contour is naturally modified with the varying contour of the cell body. The cells all contain granulae ranged upon a fibrillar ground-work, and demonstrable both in vitally stained and in fixed preparations by a variety of methods. The cells show from the first a tendency to accumulate fat, and after this has reached a certain grade, usually by the fourth or fifth day, further growth is retarded or checked. The cells may be phagocytic towards the small thymus cells.

The probable nature and origin of these cells were not easy to establish. Three possibilities suggested themselves: (1) that they were derived from connective tissue cells, either from the capsule or from the septa accompanying the blood vessels; (2) that they were derived from the endothelial cells of the capillaries; or (3) that they were outgrowths of the epithelial reticulum of the gland. The last view was the one finally adopted and for the following reasons. The growth could never be traced to the capsule of the gland when portions were incorporated in the tissue fragment. The connective tissue of the capsule showed but slight capacity for growth, only a few fibrillated spindle cells occasionally penetrating the clot for a short distance. Sometimes portions of striated muscle and connective tissue were included in the fragment, but there was never any outgrowth of

cells of this type, nor from pieces of spleen, heart muscle or intestinal wall used as controls.

That these cells were derived from capillary endothelial cells seemed improbable. The manner of their growth and their frequent origin from small masses of cells in which the absence of capillaries could be determined with certainty, seems to exclude this possibility. More positive evidence in favor of their origin from the reticular cells, is that they may, in thin portions of the culture, resemble closely the normal protoplasmic cellular framework of the gland. Although the protoplasm shows a fibrillated structure, evident especially in fixed material, there is no formation of definite fibrils upon the surface of the cells, nor do the plasmatic processes of the cells, no matter how long-drawn-out, resemble the more rigid and refractile processes of the growing connective tissue cells. In their power of phagocytizing the small cells, they function as do the normal reticular cells of the thymus. But since other cells *in vitro* may assume phagocytic powers, too much emphasis should not be laid upon this point.

If the assumption be correct that the growing cells of these cultures are derived almost wholly from the reticular cells, then we must, if we accept the prevailing view as to the histogenesis of these elements, hold them to be epithelial in nature. The epithelial origin of the thymic reticulum is believed in by all the recent workers on the structure of the thymus, including Hammar (27), Stöhr (28), Schridde (29), Maximow (30), and Cremieu (31). Dustin (32) and Pigache and Worms (33), amongst recent writers, still hold to the old view that the thymus, like the lymph-glands, has a fibrous reticulum.³ Salkind (34), in a recent paper, takes an intermediate position, claiming to have demonstrated by special methods, a fibrous reticulum analogous to that of lymph-glands, coexisting with the epithelial reticulum and giving origin to the lymphoid cells. If his views,

³ For a complete discussion of the histogenetic origin of the thymus elements, the reader is referred to the exhaustive reviews of Hammar (27) (*Ergebnisse d. Anat. u. Entwickl.*, 1910, Bd. 19, p. 1) and of Wiesel (35) (*Lubarsch and Ostertag's Ergebnisse d. Allg. Path. u. Path. Anat.*, 1911, Bd. 15, 2, p. 416).

which are counter to the prevalent conceptions of the thymic reticulum, prove correct, the interpretation of these growing elements as epithelial in nature, would be rendered less probable. The cells in question might then be derived either from the fibrous or the epithelial reticulum. Nevertheless, it is certain that in the mammalian thymus at least, it is impossible to put in evidence such a fibrous reticulum, either with Mallory's aniline blue method or with the silver impregnation method of Bielschowsky, both of which bring out with great clearness the reticular fibers of the lymph nodes. Until Salkind's work receives further confirmation, it seems unwise to revise again the current and well founded conception of the exclusively epithelial origin of the thymus reticulum.

Much emphasis has been laid by Lambert and Hanes (36) upon the differences in the character of *in vitro* growth of epithelium and connective tissue. The former tend to form solid coherent growths, while in the latter, the cells, though often connected by plasmatic processes, tend to remain isolated from one another. As a general distinction, this has undoubtedly proven to be true. The tissue which we have been studying forms at times an apparent exception since, though epithelial, it may grow either in coherent sheets of closely apposed cells, or as a loose anastomosing network, or indeed, these cells may lose their plasmatic connections with adjacent cells and wander isolated far out over the surface of the plasma. This variability in the manner of growth is not surprising if we recall the normal development of the parent tissue, and the changes which it undergoes. Beginning as a solid outgrowth of cells, the thymic epithelium early becomes rarefied into a protoplasmic syncytium in the meshes of which lie the small cells. It is probable also that under certain abnormal conditions, the reticular cells of the mammalian thymus assume a rounded form and become relatively independent of their cellular connections, often, too, exercising a phagocytic function upon the small thymic cells (Rudberg (37), Pappenheimer (7), Cremieu (31) and others). That the thymic epithelium should differ widely in the manner of its growth from the epithelium of glandular organs or of solid epi-

thelial tumors, might be expected. It is nevertheless interesting that the tissue growing under highly artificial conditions, should conform so closely to its normal type, and in the phagocytosis of the small cells, exercise a function which is characteristic of the normal thymic reticulum.

Before discussing the conclusions to be drawn from the foregoing observations, I wish to record briefly certain further studies on the comparative growth of thymus and lymph-nodes. The tissues for these experiments were obtained from young adult rats and incubated at 37°.

The small thymic cells emigrate rapidly, often reaching the edge of the plasma drop within a few hours. The lymphocytes of the lymph glands behave in the same way. Degenerative changes begin rapidly, both in the thymic cells and in the lymphocytes. The nuclei become pycnotic, fragment, and finally break up into globular, deeply staining particles. Well preserved, actively amoeboid cells were not found after forty-eight hours.

Growth from the thymic fragments begins usually on the second day, as fusiform or polygonal cells with intercellular connections. Large flat cells on the cover-glass resemble those described in the frog's thymus; but there is frequently a radially directed growth of long spindle cells resembling connective tissue. After three or four days, however, there is usually a tendency towards the formation of flat cellular planes, the growing margin of which is very definite and sharply limited. A few cells at the periphery may become partially or completely separated, but the growth on the whole is coherent (fig. 8), and often approaches in its character the growth of epithelial tissue from carcinomata, as described by Lambert and Hanes (36) and L. Loeb (38).

At this stage of the growth, moreover, the thymus culture shows changes which distinguish it definitely from the culture of lymph-node. The central fragment becomes rarefied, and there appear large numbers of globular cells of large size, which with the low power seem filled with coarse granules. These granules are really ingested small cells in various stages of pycnosis and degeneration. Scattered phagocytic cells of this type

appear among the growing cells at the margin. Where these lie against the cover-glass, they may retain their irregular shape and plasmatic processes, but the majority are globular (fig. 10). Transitions between these phagocytic cells and the growing healthy cells are evident, and their origin from the reticular cells could be definitely proven by studying serial sections of the growing tissue.

The lymph-nodes also show a growth of the fixed elements beginning usually on the second or third day, and almost invariably as radially directed sprouts. As the growth proceeds, the fragment becomes surrounded by a halo of spindle or stellate cells, which resemble in all respects, growing connective tissue cells from other organs (fig. 9). As compared with the growing reticular cells of the thymus, the cell processes are often more numerous and rather of the dendritic type with secondary branching. There is often a fibrillar differentiation on the surface of the protoplasm. The nuclei are smaller than those of the thymic cells and stain more intensely. Rarefaction of the central fragment does not occur, or at least not until a much later stage of growth. It is never so pronounced as in the thymic cultures. While it is occasionally possible to find a few lymphoid cells enclosed within a larger connective tissue cell, phagocytosis is far less conspicuous than in the thymus, and large globular cells stuffed with ingested small cells are never seen. Either the reticular cells of the thymus possess phagocytic properties which the lymph gland reticular cells do not have to the same degree; or the small thymic cells are more susceptible to phagocytosis than the lymphocytes of the lymph gland. The former explanation is the more plausible. The behavior of the small cells in thymus and lymphoid tissue, has been found to be the same in all other respects; whereas the difference in structure and origin of the reticular cells in the two tissues makes a difference in function the more probable.

CONCLUSIONS

Minute granulae of a type not hitherto described, were demonstrated in the frog's thymus, by the use of Benda's mitochondrial method. Larger gentianophile granules and droplets were found in some of the cells of this type. Whether these were secretory or degenerative in nature was not determined.

Granulae, possibly of the same nature as those demonstrable by the mitochondrial methods, were shown to be present in the living cells by the use of vital stains.

The small thymic cells also contain granulae, and in this respect, the small thymus cells are identical with the lymphocytes of the blood. This observation is in direct opposition to that of Schridde (29), that the small thymus cells, which he believes with Stöhr (20) to be of epithelial origin, may be differentiated from true lymphocytes by the absence of granulae.

In clotted plasma cultures, there is a radical difference in the behavior of the small and large thymus cells. The former show practically no capacity for further proliferation, but after a period of active motility, undergo degeneration; the latter exhibit active growth, often in the form of syncytial cell masses. They are actively phagocytic towards the degenerating small thymus cells.

This characteristic difference in the behavior of the two types of cells is opposed to Stöhr's view that the small cells are modified epithelial reticular cells and that transitions between the two normally occur.

The small cells of the rat thymus show absolutely no morphological differences from the lymphocytes of the lymph nodes; they exhibit the same active motility and the same proneness to undergo degeneration when kept in vitro.

The growth of rat thymus differs from that of lymph nodes (1) in the early rarefaction of the implanted fragment, with the appearance of numerous large phagocytic cells; (2) in the formation of tissue-like planes composed of epithelial reticular cells differing in their appearance from the fusiform or stellate cells which grow from the connective tissue capsule or reticulum of

the lymph-nodes. This characteristic difference corresponds to the different histogenesis of the thymic reticulum and suggests a different function.

In conclusion, the writer wishes to express his obligation to Dr. E. G. Kite for his kindness in supplying the vital stains used in this work, and to Dr. R. A. Lambert for his assistance in the preparation of the rat tissue cultures.

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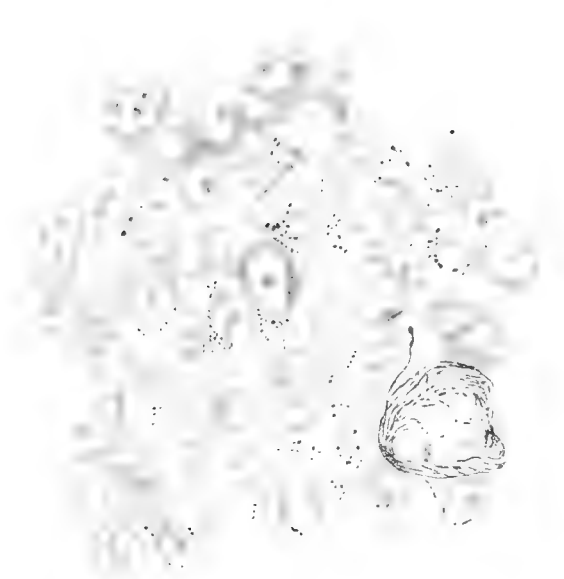
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PLATE 1

EXPLANATION OF FIGURES

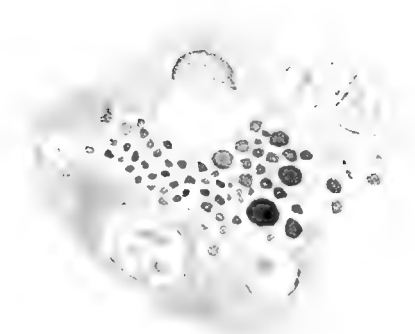
- 1 Benda fixation and stain. Mitochondria, and fibrils of myoid cell. $\times 1000$; camera lucida.
- 2 Benda fixation and stain. Granulae of small thymus cells. $\times 1000$.
- 3 Epithelial cell complex from medulla. Granulae and droplets. Benda fixation and stain. $\times 1000$.
- 4 Cell complex from medulla, containing granulae and irregular masses of gentianophile substance. Benda fixation and stain. $\times 1000$.



1



2



3



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PLATE 2

EXPLANATION OF FIGURES

5 Tissue culture, eight days. Large spindle cell with granulae and ingested small cell. Formalin, Heidenhain. $\times 1000$.

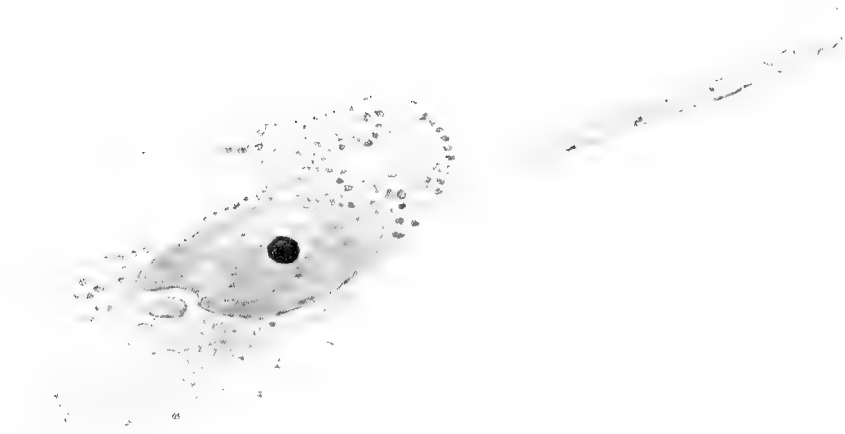
6 Tissue culture, eight days. Large reticular cell, with granulae. Formalin, Heidenhain. $\times 1000$.

7 Forty-eight hours growth in vitro. Large reticular cells on cover-glass. $\times 125$.

5



6



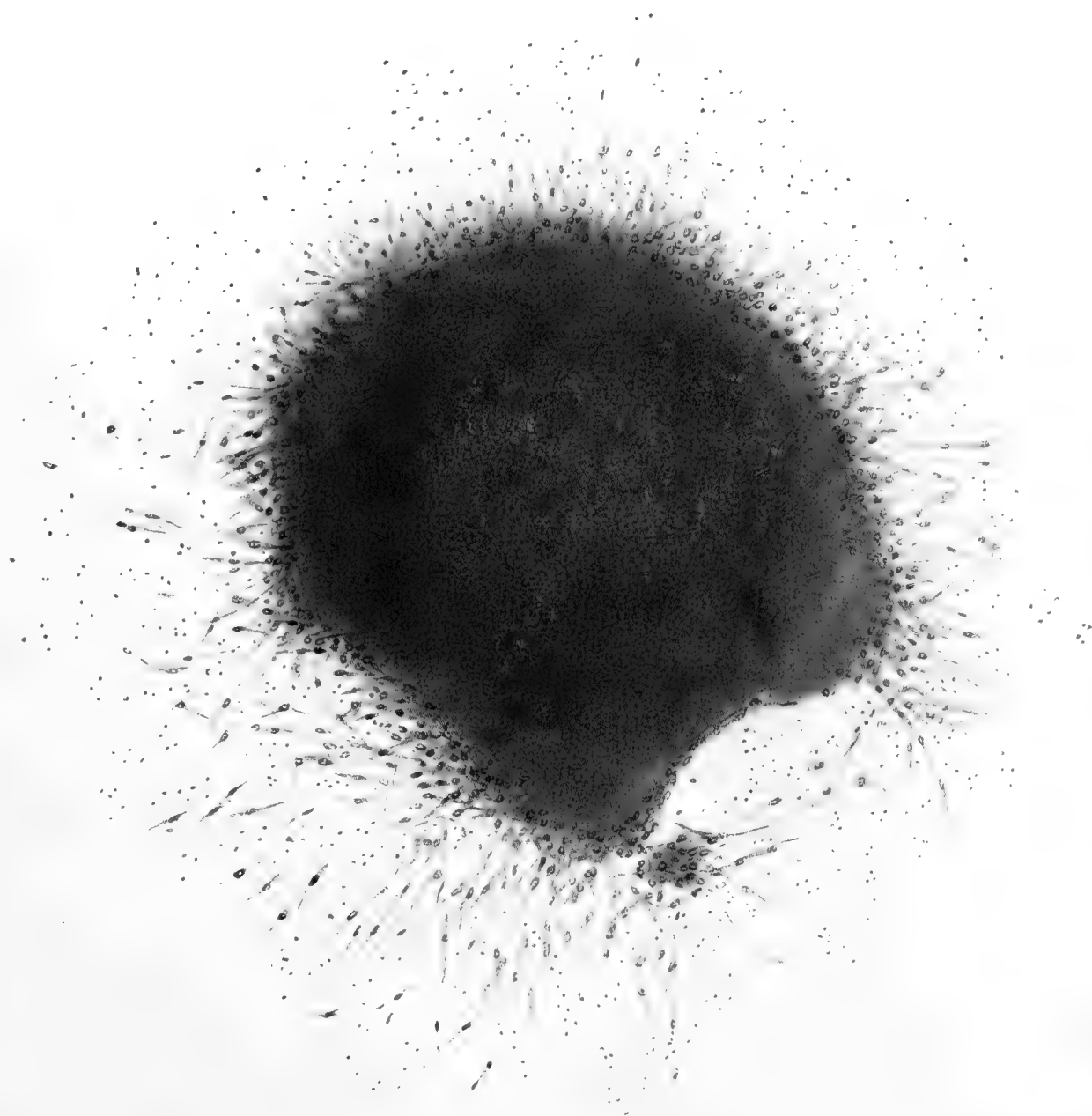
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EXPLANATION OF FIGURE

8 Rat thymus; four days growth in vitro



EXPLANATION OF FIGURE

9 Rat lymph gland; four days growth in vitro



EXPLANATION OF FIGURE

10 Rat thymus. Large reticular cell, filled with ingested small thymus cells and nuclear fragments.

THE DEVELOPMENT OF THE ELASMOBRANCH LIVER

I. THE EARLY DEVELOPMENT OF THE LIVER

II. THE DEVELOPMENT OF THE LIVER DUCTS AND GALL-BLADDER

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FIFTY-FOUR FIGURES

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I. INTRODUCTION

The development of the elasmobranch liver offers many problems of interest not only for themselves, but because of their bearing upon questions regarding the structure of this organ in the vertebrates in general. The hepatic duct system shows here in its earlier stages a number of characters which are masked in forms which are more complicated or which undergo a more rapid development and in later stages the organ exhibits a number of peculiarities of interest in the light of recent work on the relation of parenchymous to vascular structures.

The present paper, based upon a study of an extensive series of embryos of *Squalus acanthias*¹ gives an account of the early development of the liver and the history of the principal liver ducts and the gall bladder. In a later section it is hoped to give an account of the development of the smaller rami of these ducts and of the hepatic parenchyma.

II. LITERATURE

At this point I shall review only briefly the literature of the general development of the elasmobranch liver. Particular points are considered in more detail in the separate sections and reviews of the literature by Brachet ('97), Choronschitzky ('00), Piper ('02), and Weber ('03) already cover a part of the subject.

In common with so many points in selachian embryology there was but little knowledge of the development of the liver until the researches of Francis Balfour. Rathke ('27) published an account of several selachian embryos including one of *Squalus mustelus* (*Mustelus canis*?) of an approximate length of 45mm., in which he described the division of the liver into an anterior mass and two posterior lobes and traced the course of the ductus choledochus to the intestine. He stated that the gall bladder was absent in this specimen as well as in an older one of *Squalus canicula* (*Scyllium canicula*?). At such a stage this structure is, in fact, embedded in the liver substance and not visible externally. Rathke observed the gall bladder however in an embryo of *Squalus mustelus* 7 inches 2 lines in length, and traced the course of the vitelline veins to the liver and followed their ramifications in this organ to their final connections with the sinus venosus.

Franz Leydig ('52) in his "Beitrage zur mikroskopischen Anatomie und Entwicklungsgeschichte der Rochen und Hai"

¹ This material consisted of sectioned embryos of *Squalus acanthias* from 3 to 86 mm. in length as well as several specimens of the same species in the 'pup' stage, and a few large embryos of *Mustelus laevis* and *Squalus sucklii* (?). In a large part these specimens were from the Harvard Embryological Collection, and I wish to express here my thanks to Dr. Charles S. Minot for their use for a prolonged period, as well as for the privileges of his laboratory during a part of the time while this study was in progress. Four specimens were also from the embryological collection of the University of Kansas. For their use I am indebted to Dr. C. E. McClung.

figured a cleared embryo of *Squalus acanthias* approximately 18 mm. in length in which the liver is pictured as a dark irregular mass lying just posterior to the heart. He described the cells of this organ as arranged in lobules and containing numerous fat droplets embedded in a homogeneous ground substance. He also traced the course of the omphalo-mesenteric veins through the liver.

Balfour ('76) working with *Scyllium*, *Pristiurus* and *Torpedo*, described the liver as arising in Stage I, when forty-eight pairs of somites and three pairs of gill pouches are present, as a ventral outgrowth from the 'duodenum' directly anterior to the umbilical canal. This outpouching gives off at once two lateral diverticula which are the rudimentary lobes of the liver, while the remainder of the original ventral median pouch forms the gall bladder and ductus choledochus. The hepatic tubule diverticula appear as hollow buds by stage K, and increasing rapidly both in length and number soon anastomose forming a regular network. In the course of these changes the lumina of the tubules become much reduced in size. The gall bladder arises as dilatation of the anterior end of the median pouch and its duct joining with the hepatic ducts forms the ductus choledochus.

Hammar ('93) figured and described the first series of reconstructions of the selachian liver. These are of embryos of *Torpedo ocellata*, the first of forty segments, and the remainder, 9, 11, 15 and 18 mm. long respectively. These specimens correspond roughly to Balfour's stages J, K, L, M, and O. From the study of these models Hammar concludes that the liver arises primarily from three diverticula, two lateral and one median in position, and not from a single median and ventral pouch as stated by Balfour. He also considered the liver proper to arise from the lateral diverticula, the median giving rise to the gall bladder and its duct only. He noted further that there was a twisting of the fore gut from left to right, a point apparently overlooked by other observers. The account of the formation of the common bile duct and gall bladder is fairly complete, but he traced the hepatic ducts no farther than their entrance into the lateral masses of hepatic trabeculae.

In a later paper of a more general nature on the early developments of the liver, Hammar ('97) expresses his views in regard to the origin of the selachian liver as follows:

Bei den Selachiern wird ebenfalls eine unter dem Herzen hervorragende stufenähnliche Leberfalte gebildet, an deren cranialen Rand schon frühzeitig *zwei bilateral-symmetrische Divertikel* auftreten—Zwischen diesen beiden Divertikeln und beinahe gleichzeitig mit ihnen entsteht als eine cranioventrale Verlängerung der Leberfalte noch ein drittes medianes Divertikel, aus welchen die Gallenblase und Gallenblasengang hervorgehn.²

I quote Hammar at length for he holds a view somewhat different from that accepted by most investigators and one which this paper will in part confirm.

Laguesse ('94) gave a brief account of the development of the liver in *Squalus acanthias* in connection with his study of the pancreas in this form. He states that the liver arises a little later than the pancreas, a point which has since been disproven, and although in possession of younger embryos, he apparently first observed the organ in an embryo 8 mm. in length, where it appeared as a thick walled ventral pouch extending from the primitive sinus venosus to the anterior wall of the yolk-stalk. An embryo of 9 mm. length showed the formation of the lateral diverticula. At 16 mm. the buds of the hepatic tubules had appeared and at 19 mm. they were fused together, forming the typical net-work of hepatic trabeculae so often described. Laguesse emphasizes the late appearance of the gall bladder as a structure distinctly separated from the hepatic anlage proper. This seems to me to be a point of much importance which apparently has not been recognized by other workers in this field, with the exception perhaps of Hammar.

Brachet ('96) devoted the first part of his contribution to the development of the liver and pancreas to the selachian liver as represented by *Torpedo ocellata*. Like Hammar he presented a series of reconstructions corresponding to Balfour's stages J, K and L. The two main points in this paper consist of, first, an affirmation of Balfour's statement that the liver arises between

² The italics are the author's.

the anterior intestinal portal and the sinus venosus as a single median ventral pouch from which the lateral pouches secondarily arise, and second, the recognition of the fact that the median pouch which can be distinguished when the liver reaches the rinvaginated stage consists of two portions, named in accordance with the nomenclature suggested by Goeppert ('93) the 'pars hepatica' which lies anteriorly and joins with the two lateral pouches in forming the true hepatic parenchyma, and a posterior portion called the 'pars cystica' which forms the gall bladder and the cystic and common bile ducts. Brachet followed the history of the latter structures in detail, but gives little information as to the history of the hepatic ducts, although he recognized that they were formed from the bodies of the lateral pouches and considered that these pouches were reduced in caliber in the course of their transformations. In the *Ergebnisse* for the same year Brachet ('97) repeats his conclusions and summarizes the preceding literature since the time of Balfour.

Rückert's work ('96) on the development of the spiral valve in *Pristiurus* is illustrated by three reconstructions, two of which include the gall bladder and ductus choledochus and illustrate well the forward migration of the former structure. Rückert describes the migration of the ostium of the ductus choledochus in relation to the vitelline duct and its movement from left to right along with the spiral valve in later stages.

Mayr ('97) in his account of the development of the pancreas in *Pristiurus* and *Torpedo* incidentally describes the condition of the liver in several embryos. His description coincides with that of Balfour and Brachet, but he emphasizes the fact that in early stages the pars hepatica is single anteriorly and that the two lateral pouches diverge from the median line as they extend backward.

Choronschitzky ('00) published a paper of some magnitude, describing the development of the liver and certain other viscera in all classes of vertebrates. *Torpedo* was employed as a representative of the fishes. He gave an account of four stages of this form, the youngest being one in which the liver consisted of a median and two lateral pouches from which four 'secondary'

pouches sprang and the oldest one in which the liver had reached a length of 1.1 mm. as measured from transverse sections and was a solid parenchymous organ. He traced the separation of the liver from the gut and the formation of the gall bladder in some detail and followed out in a general way the development of the two larger hepatic ducts. On the whole his description is a confirmation of that of Brachet. The reconstruction method was apparently not employed.

The work of Debeyre ('09) is primarily a study of the origin of the hepatic cylinders. However, working on Laguesse's *Acanthias* material, he confirms that author's account of the early stages of the liver, including the location of the gall-bladder anlage in the extreme posterior end of the hepatic diverticulum.

The papers of Braus ('96), Holm ('97), and Minot ('00) deal mainly with the histogenesis and vascularization of the liver, but contain incidental references to its early development. Braus and Minot accept Balfour's account as essentially correct.

III. DEVELOPMENT OF THE LIVER IN EMBRYOS FROM 3 TO 10 MM. IN LENGTH

In *Acanthias* the first evidences of liver development are to be seen in embryos of 19 to 21 segments. At this stage, which is a little younger than No. 16 of the Normal plate series and lies between Balfour's stages G and H, the embryo shows a distinct head bend and the medullary canal is closed except for the large netropore. The archenteron which is still in quite a primitive condition is outlined in figure 1, a graphic reconstruction from transverse sections. The fore gut is separated from the entoderm of the blastodisc and is approximately one-sixth the length of the body. The lateral walls of the mid and hind gut are still separated at their bases almost throughout by a considerable ventral cleft, and meet only a few sections anterior to the tail to form the lower and hinder walls of the neurenteric canal. The first gill pouch is a shallow depression, present on one side only. The second gill pouch is indicated by a very slight depression in the dorsal part of the lateral wall of the pharynx immediately pos-

terior to the first pouch. Its ventral portion is not as yet evaginated.

As the fore gut approaches its connection with the blastodermic entoderm it is at first broadly ovoid in cross section with the narrower end of the oval upward and its vertical diameter increases posteriorly. Back of the fore gut the archenteron is flattened transversely until it is little more than a high, narrow fold of entoderm, the transverse diameter of which is less than one-fourth of the vertical diameter. The lateral walls of the gut for a short distance behind and also a little in front of the point of union of the fore gut and the mid gut are differentiated into dorsal and ventral zones. The epithelium of the dorsal zone is

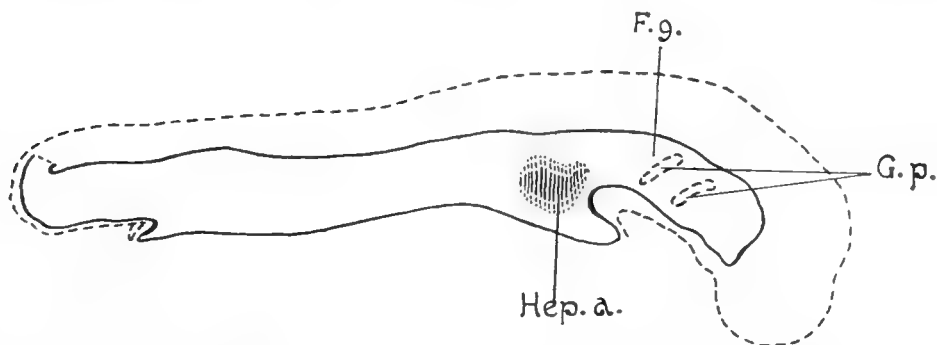


Fig. 1 Lateral view of the archenteron of an embryo of 19 to 20 segments, 4.0 mm. in length (H.E.C. 930). $\times 20$. *F.g.*, fore gut; *G.p.*, gill pouches; *Hep.a.*, hepatic area.

from 25 to 30 μ in thickness and contains two or more rows of more or less interlocking oval nuclei. This is the primitive condition found throughout the walls of the archenteron, both dorsally and ventrally in earlier stages. Close to the lower border of the dorsal zone there is on either side a shallow and not always continuous longitudinal groove which later becomes a definite and important landmark. For these I suggest the name para-archenteric grooves. The ventral zone in this region, as is seen from following its later history, represents the liver anlage. Here the epithelium is approximately half as thick as that of the dorsal zone, and the nuclei, which form a single row only, lie in the basal portion of the epithelium. Ordinary stains do not bring out definite cell walls in either the dorsal or ventral zones at this stage. There

are no definite boundaries, except the dorsal one to the hepatic area at this time. The arrangement of nuclei just described extends ventrally nearly to the point where the lateral walls of the archenteron turn laterad as a part of the blastoderm. Longitudinally the hepatic region extends forward a little past the posterior end of the fore gut to become indistinguishable in the general ventral enlargement of the pharynx already referred to. Its characteristics are less noticeable as we follow the gut posteriorly and $100\ \mu$ behind the point of union of fore and mid gut the hepatic area is indistinguishable from the other entoderm. There is but a slight indication of evagination of the liver area. Although the lumen between the walls of the ventral zone is nearly twice as wide as that above, this width is due mainly to the decrease in the thickness of the walls themselves, the entire transverse diameter of the gut being a little greater dorsally than ventrally.

A slightly older embryo having 24 trunk segments and 3.6 mm. in length, which is a little more advanced than the Normal plate No. 20 (Scammon '11), gives a clearer picture of the liver anlage. The pharynx, from which two well formed gill pouches project and fuse with the skin ectoderm, is followed by a short segment of gut which represents both the oesophagus and the anterior part of the stomach. This segment is elongately oval in cross section with very much thickened lateral walls. It is somewhat produced ventrally as it approaches the anterior wall of the yolk-stalk, and in this ventral region shows lateral expansion. The archenteron extends forward forming a large anterior recess above the yolk in front of the anterior wall of the yolk-stalk. Immediately behind the point of union of the fore gut with the yolk-stalk the archenteron has the same form as in the younger embryo, being greatly elevated and flattened transversely. The distinction between dorsal and ventral zones is fairly marked, and the para-archenteric grooves can be traced along the gut above the hepatic region, although in places they are very faint and shallow. The ventral zone in this region is now distinctly curved outward, forming a pair of shallow, lateral diverticula which extend approximately $100\ \mu$ posterior to the anterior vitello-

intestinal junction. The walls of these lateral diverticula are of the same thickness as those of the dorsal zone, i.e., 25 to 30 μ , but the nuclei are somewhat more elongated and lie at the basal ends of the cells, leaving a clear zone towards the lumen of the gut. The boundaries of the cells are faintly distinguishable.

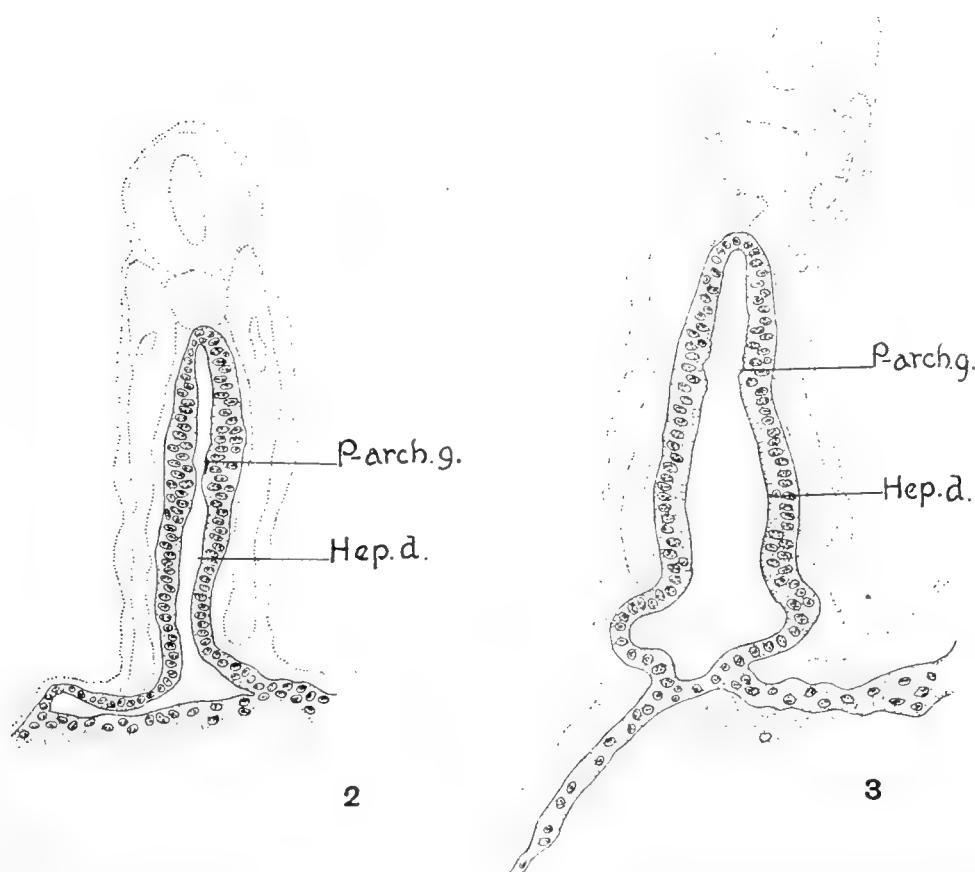


Fig. 2 Transverse section of an embryo of 19 to 20 segments, 3.0 mm. in length (K.U.E.C. 451). 0.06 mm. posterior to the anterior wall of the yolk stalk. $\times 100$. *Hep.d.*, hepatic diverticulum; *P-arch.g.*, para-archenteric groove.

Fig. 3 Transverse section of an embryo of twenty-four segments, 3.6 mm. in length (S.C. 14). 0.09 mm. posterior to the anterior wall of the yolk stalk. $\times 100$. *Hep.d.*, hepatic diverticulum; *P-arch.g.*, para-archenteric groove.

Above and below the diverticula the nuclei are broadly oval and scattered through the thickness of the epithelium, and there are no distinct cell outlines to be seen with ordinary stains. Figure 3 is a transverse section of this embryo 90 μ behind the anterior wall of the yolk-stalk.

A distinct step in development is shown by an embryo 5 mm. in length (K.U.E.C. 449³). This embryo has segments and three gill pouches, none of which open to the exterior. The gut is still connected with the yolk entoderm by a yolk-stalk, which has an antero-posterior diameter equal to nearly half the length of the archenteron. A lateral view of the liver region is outlined in figure 4, which is a graphic reconstruction, with the hepatic area outlined with broken lines. The lateral hepatic diverticula are somewhat better defined, both dorsally and ventrally, than in the preceding stage and are somewhat deeper. They show in a more marked way the histologic differentiation described for the preceding embryo, as is illustrated by figures 5 and 6, for the cells have lengthened so rapidly that epithelium is one-fourth to one-half thicker than that of the dorsal zone of the gut above it. Numerous mitoses indicate the rapid growth now taking place in this region. It will be noticed from figure 4 that the lateral liver diverticula now extend forward distinctly beyond the anterior wall of the yolk-stalk. Here the right and left pouches are fused, forming a single median and ventral pouch in the posterior part of the fore gut. The finer structure of this anterior part is illustrated in figure 5. It shows the same character and distinction from the remainder of the gut wall as does the posterior part of the anlage. The right and left omphalo-mesenteric veins are now present, although of small caliber.

A slightly older embryo 6.4 mm. long shows somewhat the same stage of development and the liver region has been reconstructed in wax. Figures 30 and 31 show anterior and right lateral views of this object. The entire embryo is inclined markedly to the left. The fore gut, ovoid in cross section, becomes immediately flattened and triangular after passing the anterior wall of the yolk-stalk. The lateral hepatic diverticula are distinctly outlined and fuse together, forming anteriorly a ventral pouch in the floor of the fore gut. The antero-posterior length of the hepatic anlage

³ The following designations of embryos are employed in this paper: H.E.C. = Harvard Embryological Collections. K.U.E.C. = Kansas University Embryological Collection. S.C. = Author's Collection.

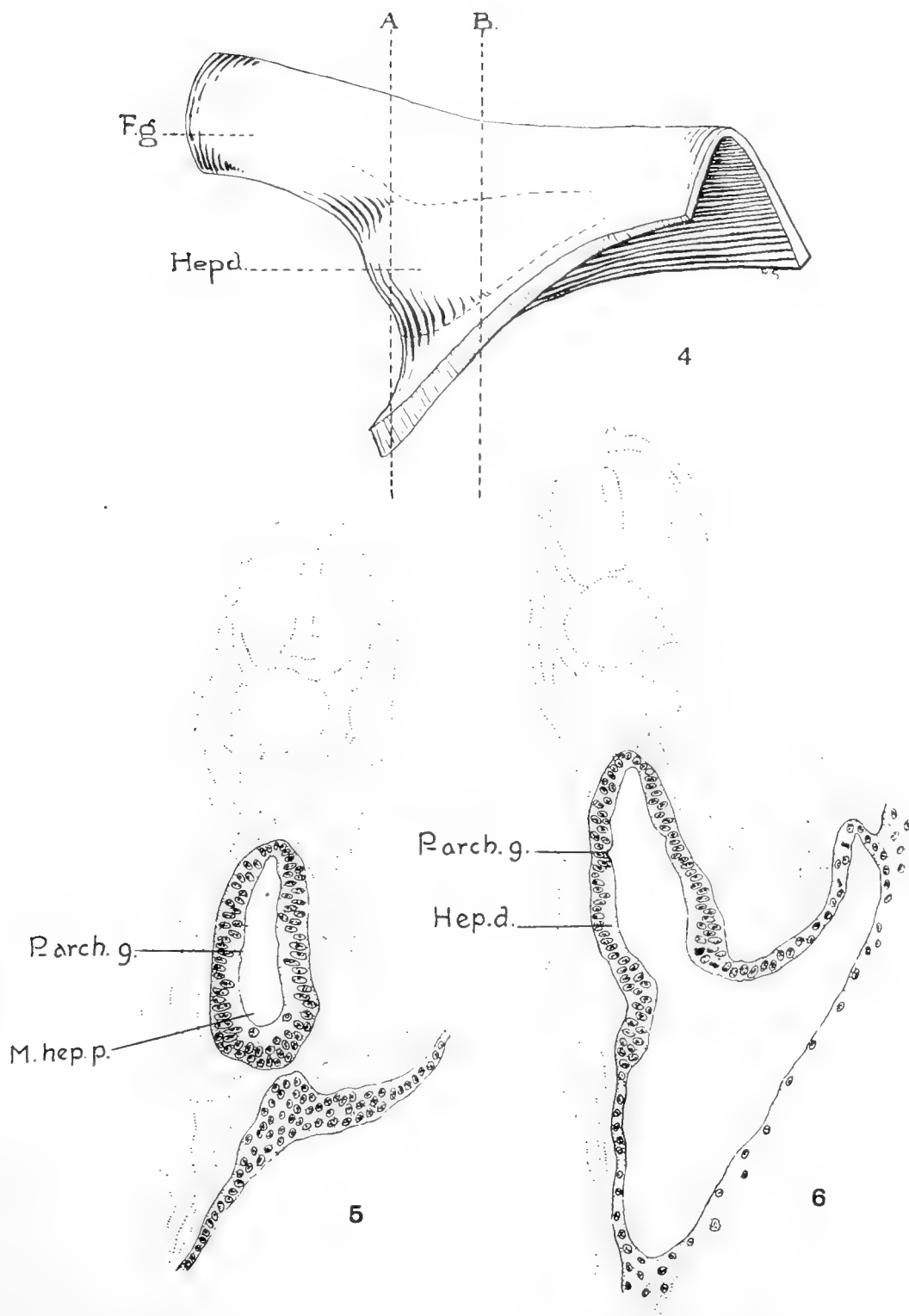


Fig. 4 Graphic reconstruction of a portion of the fore and mid gut of an embryo 5 mm. long (K.U.E.C. 449). $\times 50$. The dotted lines indicate the borders of the hepatic thickening. Lines A and B indicate the planes of sections represented in figures 5 and 6. *F.g.*, fore gut; *Hep.d.*, hepatic diverticula.

Fig. 5 Transverse section of the same embryo 0.03 mm. in front of the anterior wall of the yolk stalk. $\times 100$.

Fig. 6 Transverse section of the same embryo 0.06 mm. posterior to the anterior wall of the yolk stalk. $\times 100$. *Hep.d.*, hepatic diverticula, *M.hep.p.*, anterior-median hepatic pouch, formed by the fusion of the lateral hepatic diverticula. *P-arch.g.*, para-archenteric grooves.

is 0.30 mm. The length of the fused anterior parts of the lateral diverticula is 0.13 mm., while that of the still separated posterior portions is approximately 0.17 mm. The posterior part of the lateral liver anlagen, however, can hardly be called diverticula, as they are little more than thickened plates of cells. The histologic differences between the liver diverticula and the remainder

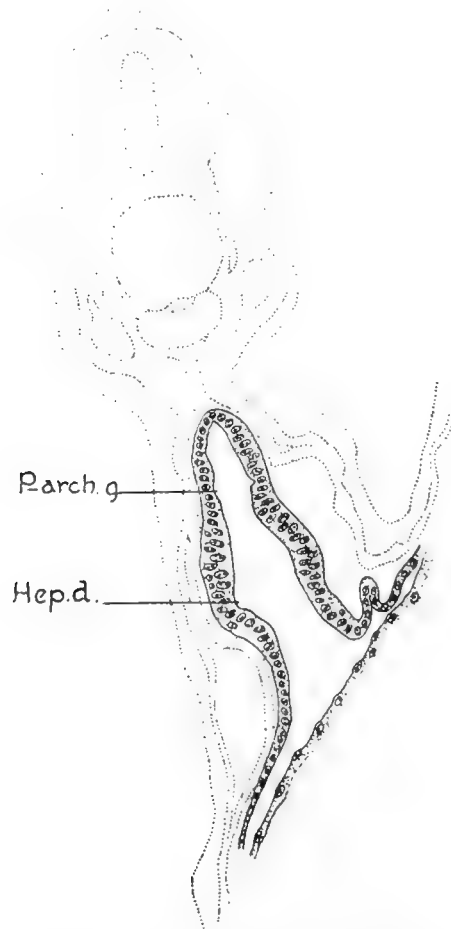


Fig. 7 Transverse section of an *Acanthias* embryo 6.4 mm. long (S.C. 19), 0.05 mm. posterior to the anterior wall of the yolk stalk. $\times 100$. *Hep.d.*, hepatic diverticula; *P-arch.g.*, para-archenteric groove.

of the archenteron walls are shown in figure 7. The nuclei in the walls of the lateral diverticula are no longer arranged in a single layer, but are irregularly placed in the basal halves of the cells. Their elongation is noticeable. The cytoplasm of the cells of the hepatic area is condensed and stains darkly as compared with that of the cells above. The para-archenteric grooves are very

broad and shallow and can be followed with difficulty above the lateral diverticula.

Two marked changes now take place in the liver anlage, bringing about the condition described by Brachet ('96) and others as the primitive one. These are the appearance of the gall bladder and the distinct lateral and dorsal evagination of a part of the lateral hepatic diverticula. An embryo 7 mm. in length (H.E.C. 752), which is probably a little younger than No. 21 of the Normal plate series and a little older than Balfour's Stage H is the earliest specimen in which I have observed any indication of the gall bladder. Both the separated posterior parts and the anterior median pouch, formed by the fusion of the anterior ends of the lateral hepatic diverticula, are more pronounced than in the embryo just described. The anterior wall of the yolk-stalk is much thickened over its entire extent, but particularly just below the point where it becomes continuous with the floor of the fore gut. The gall bladder is represented by a very shallow median depression at this place. Numerous mitoses indicate that the epithelium is growing rapidly in this region. The early evagination of the gall bladder must take place with some rapidity, as it is very difficult to find any specimens between the stages when this structure is entirely absent and when it is a deep, well-marked pouch.

A specimen which illustrates both the early development of the gall bladder and the growth of the lateral diverticula, and is quite comparable with the first members of Hammar's ('93) and of Brachet's ('96) series of models, has been reconstructed and figures 33 and 34 are anterior and left lateral views of the model. This specimen, which is 7.5 mm. in length (H.E.C. 1503 and No. 24 of the Normal plate series), has 54 to 55 trunk segments and four gill pouches, two of which open exteriorly. The spiral valve makes one and one-third turns of the gut. The distance from the last (fourth) gill pouch to the anterior wall of the yolk-stalk is approximately one-fourth of the complete length of the alimentary canal and about equal to the antero-posterior diameter of the yolk-stalk. The lateral hepatic diverticula are differentiated into three parts. Anteriorly they are fused, form-

ing the single median pouch already mentioned which now projects downward from the floor of the fore gut. Continuous with the median pouch thus formed are the middle parts of the diverticula which are expanded laterally and dorsally and which will be referred to hereafter as the lateral hepatic pouches. The lateral hepatic pouches extend backward as far as the anterior wall of the yolk-stalk, and there become continuous with the posterior parts of the lateral diverticula which remain almost unchanged from their slightly expanded condition of earlier stages, and which will be referred to as the pars ductus, as it is from them that the major portion of the ductus choledochus is formed. The left lateral pouch becomes continuous with the pars ductus of that side without any distinct line of demarcation. On the right side, however, the pouch ends abruptly by projecting nearly at right angles from the gut wall. Deep grooves, of which the left is the more pronounced, intervene between the latter walls of the ventral part of the fore gut and the mesial walls of the dorsally growing lateral pouches. These indicate the beginning of the process by which the liver will be eventually cut off from the gut tube above. The gall bladder is present as a deep ventral pouch, lying between the median liver pouch in front and the anterior wall of the yolk-stalk behind. Its walls are directly continuous with the ventral part of the liver pouch and the pars ductus above, but a slight longitudinal groove marks the boundary between the structures. This groove becomes deeper as it proceeds anteriorly until a point is reached about one-fifth of the length of the pouch from its anterior wall. Here the groove is entirely absent and there is thus left a small anterior expanded segment of the gall bladder stalk which is the anlage of the primitive cystic duct. A point worth emphasis is that the entire liver anlage shows a slight rotation to the left and that the left lateral liver pouch shows a greater dorsal growth than does the right. The para-archenteric grooves have remained unchanged.

The changes which now follow are those of passive growth. The lateral pouches expand transversely and become almost globose in outline. At the same time there is a slight growth

dorsally which deepens the groove between them and the gut mesially. The pars ductus expands somewhat and becomes more sharply marked off from the archenteron posteriorly. The anterior median pouch also shares in this expansion but shows no other changes. Likewise the gall bladder becomes rotund, a distinct groove intervenes between its dorsal-anterior angle and the liver anlage and a ventral notch of some depth separates the sack from the anterior wall of the yolk-stalk behind. In front of this ventral notch the sac is still continuous with the liver anlage proper, but the longitudinal construction between the two structures mentioned in the description of the preceding embryo is present in a more distinct form. These changes are illustrated in figures 35 and 36, of a wax reconstruction from an embryo 9 mm. in length, the general anatomy of which has been previously illustrated in graphic reconstruction in figure 11 of the Normal plates of *Acanthias*.

A little later, as shown in an embryo of sixty somites with three open and two closed gill pouches and two complete turns of the spiral valve, the lateral pouches lose their expanded outline, and becoming flattened laterally, enter upon a decided dorsal growth (figs. 37 and 38). At the same time their posterior margins become sharply differentiated so that they extend out from the gut at an abrupt angle and their distal edges show several slight irregularities. The anterior median pouch remains practically unchanged. The lateral grooves along which the liver eventually separates from the fore gut above it are now considerably deepened and extends the entire length of the line of attachment of the liver evagination, although they are still shallow anteriorly. The left lateral pouch bears on its lateral surface three small longitudinal ridges. These together with the dorsal irregularities mentioned above constitute the anlagen of hepatic tubules and will be discussed in the section dealing with these structures. The gall bladder while no larger than in the preceding embryo is separated from the anterior wall of the yolk-stalk by a deeper ventral notch and the constriction between the sack and the median part of the liver above is more pronounced. Both this

and the preceding stage show a slight but distinct rotation of the anterior part of the liver to the left around the fore gut as an axis.

A considerable advance in development is seen in an embryo only a millimeter longer than the preceding one. This specimen corresponds fairly well with Balfour's stage I, or No. 24 of the Normal plate series the embryos of which measured 11.5 mm. It has sixty-five segments, three open gill slits and two unopened gill pouches and four turns of the spiral valve. A reconstruction of the liver and adjoining archenteron is illustrated in figures 28,

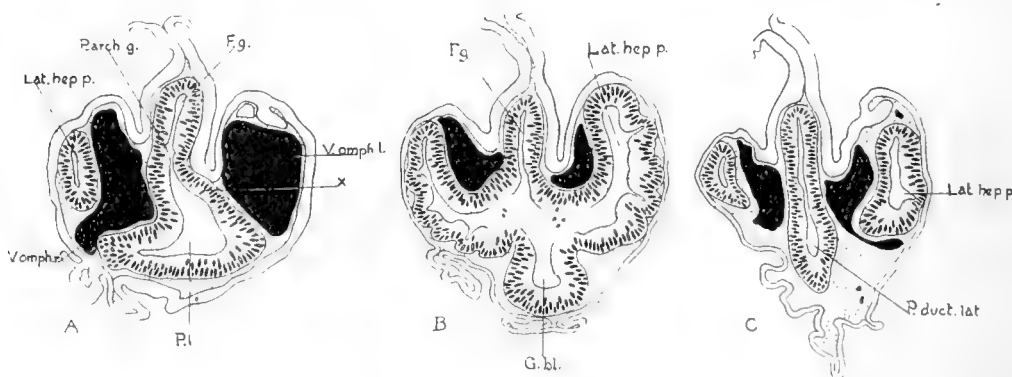


Fig. 8 Three transverse sections through the liver region of an *Acanthias* embryo 10 mm. long (S.C. 20). $\times 50$. A, Through the anterior part of the median hepatic pouch (pars hepatica mediana). B, Through the posterior part of the median pouch (pars ductus mediana). C, Through the gut just posterior to the liver proper showing the pars ductus lateralis. F.g., fore gut; G.bl., gall bladder; Lat.hep.p., lateral hepatic pouch; P-arch.g., para-archenteric groove; P.duct.lat., pars ductus lateralis; P., pars hepatica mediana; V.omph.l., V.omph.r., left and right omphalo-mesenteric veins; X, anlage of the ductus choledochus.

39 and 40. The process by which the hepatic pouch will eventually be separated from the gut above is well under way. The median hepatic pouch from which the lateral pouches spring and to which the lateral pouches are attached is rather triangular in cross section anterior to the origin of the lateral pouches. The broader part is below and the narrow dorsal extremity joins the floor of the fore gut anteriorly a little to the right of the median line (fig. 8 A).

The ventral part of the gut is also rotated to the right so that these two structures join at an oblique angle thus forming a broad

shallow groove on the left hand side while on the right they form a smooth somewhat convex surface. Posteriorly the median hepatic pouch is continuous with a short segment of the gut which in turn becomes attached to the yolk stalk. The para-archenteric grooves are still distinguishable and mark the plane of union between the hepatic anlage and the gut (fig. 8, *A*, *B* and *C*, *P-arch. g.*). Two parts can be distinguished in the median pouch, an anterior one which projects a little in front of the anterior end of the attachment of the lateral pouches, and a posterior part which is directly continuous with the former and from which the lateral pouches and the gall bladder take origin. Although these divisions are not sharply marked off at present, they later become quite distinct. The anterior one from its later history may be called the *pars hepatica mediana* because it shares with the lateral pouches or *pars hepatica lateralis* in the formation of hepatic ducts and trabeculae. The posterior later develops into a part of the ductus choledochus and may be called the *pars ductus mediana* in distinction to the anterior and to the *pars ductus lateralis* formed from the posterior portions of the original hepatic diverticula. The upper surface of the posterior part or *pars ductus mediana* of the median pouch lying on either side of this dorsal connection with the gut already shows a peculiar modeling indicative of the course which will be eventually taken by the ductus choledochus (fig. 28). There is a marked expansion which extends from the right anterior angle of the median pouch obliquely backward to the posterior left corner. The anterior, i.e., right portion of this swelling is the more marked. From the posterior edge of the middle pouch this expansion is continued backward into the mid gut as a symmetrical lateral expansion of the ventral part of the 'connecting piece' between the hepatic and stomach anlagen above it to the yolk-stalk and overlying gut. As seen from their position these lateral expansions are the remains of the posterior ends of lateral hepatic diverticula or *pars ductus lateralis* (fig. 8, *C*). Thus there can already be recognized two distinct parts of the ductus choledochus; an anterior asymmetric portion and a posterior part which is symmetrically placed.

The lateral pouches have continued their dorsal growth and now extend as far upward as the dorsal surface of the gut. Their upper parts are expanded, particularly posteriorly, so that a proximal constricted stalk and a distal expanded portion can be distinguished. In earlier stages as shown by figures 33 and 37, the origin of each lateral pouch was continuous with the entire lateral edge of the median one, but at this stage it is confined to the posterior four-fifths of this edge. The dorsal part of each lateral pouch is curved a little medially. All the external lateral surface of each lateral pouch is corrugated with rather irregular longitudinal ridges which are somewhat broken by shallow transverse fissures (fig. 40). Similar ridges are forming on the ventral surface of the median pouch anterior to the gall bladder. The dorsal edge of each median pouch is also rendered exceedingly irregular by the several small pouches springing from it. All of these structures are the anlagen of hepatic tubules the formation of which was referred to in the description of the preceding embryo.

The gall bladder is now a large thick-walled sac, ovoid in shape and somewhat flattened dorso-ventrally. Distinct grooves separate it from the median liver pouch and connecting piece behind and along its dorsal edge. These grooves are however deeper posteriorly than anteriorly as might be expected from their history in earlier stages. The extreme anterior tip of the gall bladder is drawn to a point and projects very slightly forward below the anterior part of the median liver pouch.

IV. CONCLUSIONS

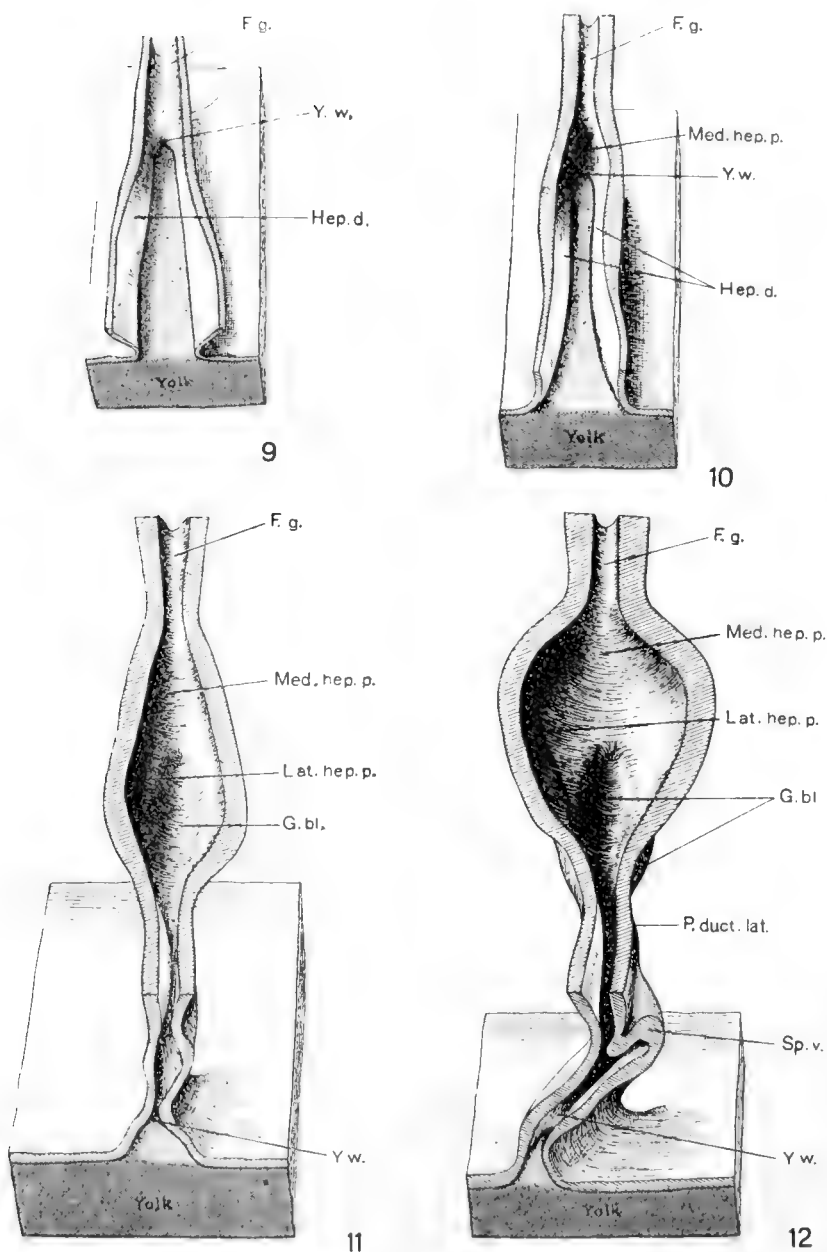
All the main divisions of the liver are now established and before giving an account of their later history it may be well to summarize the development of the organ up to this stage. The semi-diagrammatic models shown in figures 9 to 12, illustrate this process. They are of embryos 3.6, 6.4, 7.5 and 9 mm. long respectively, and are based upon wax reconstructions and measurements of specimens described in the preceding pages. In each a portion of the mid and fore gut is represented as resting on a block of yolk. The dorsal half of the archenteron is cut away

so that one looks down on the interior of the ventral half of the gut from above and a little behind. Figure 9 shows the liver as a pair of shallow lateral diverticula, lying mainly behind the point of union of fore gut and blastodermic entoderm or the anterior wall of the yolk-stalk. As the embryo is elevated and farther separated from the blastoderm the anterior wall of the yolk-stalk retreats posteriorly. This brings the anterior ends of the lateral diverticula in contact and they fuse more and more, forming the median liver pouch and producing the condition shown in figure 10.

Here the liver anlage is *U*-shaped with the limbs of the *U* turned posteriorly and slightly divergent. The fusion of the lateral diverticula is continued along with the posterior progression of the anterior wall of the yolk-stalk. At the same time parts of these structures undergo unequal growth. In each diverticulum the middle part above and a little behind the fused median portion begins a rapid dorsal and lateral growth, producing the structures known variously as 'lateral pouches' 'ébauche hépatique,' and 'Seitendivertikel.' The posterior parts of the primitive lateral diverticula, or pars ductus, which extend backward to or beyond the anterior wall of the yolk-stalk share but little in this growth, but remain unchanged in their primitive condition as a pair of shallow lateral diverticula until at a much later period, they are transformed into a part of the ductus choledochus.

In the meantime the gall bladder arises as an out-pouching of the dorsal part of the anterior wall of the yolk-stalk and being somewhat cut off from that structure by the posterior growth of the fore gut comes to lie between it and the median liver pouch anteriorly and with the lateral median pouches bounding its sides. This stage is represented in figure 11. Figure 12 shows a somewhat later stage modified from the preceding by the greater expansion of all parts of the hepatic structure and by a still greater elongation of the fore gut.

This account of the development of the liver is to some extent in accord with that given by Hammar ('93) as opposed to the idea of a single median ventral anlage as advanced by Balfour ('76), Laguesse ('93), Brachet ('96) and Choronschitzky ('00).



Figs. 9, 10, 11, 12 A series of semi-schematic reconstructions to illustrate the early development of the liver; all $\times 50$. The plan of reconstruction is explained in the text on p. 350. *F.g.*, fore gut; *G.bl.*, gall bladder; *Lat.hep.p.*, lateral hepatic pouches; *Med.hep.p.*, median hepatic pouch; *Hep.d.*, hepatic diverticula; *P.duct.lat.*, pars ductus lateralis; *Sp.v.*, spiral valve; *Y.w.*, anterior wall of the yolk stalk.

Fig. 9 Embryo 3.6 mm. long.

Fig. 10 Embryo 6.4 mm. long.

Fig. 11 Embryo 9 mm. long.

From a short study of early *Torpedo* embryos I am inclined to think that in this form the stage in which the liver exists as a pair of lateral diverticula must be very brief if at all present, because embryos of this form separate from the blastoderm at an earlier period than do *Acanthias* embryos and have a comparatively small yolk-stalk. The rapid formation and elongation of the fore gut accompanying these changes may involve the hepatic areas before they are differentiated as pouches. As regards *Acanthias* and probably other *Selachii*, it appears to me very probable that investigators have been misled from a study of embryos which have advanced to a considerable extent in the process of development. It is interesting to note that in embryos of other groups of animals possessing large yolk-laden ova the liver forms at a stage when other organs are in quite a primitive condition, and in only slightly teleolecithal ova the time of origin is still younger. At the time when Balfour, Brachet and Laguesse record the appearance of the liver in elasmobranch fishes the embryo is well established, several gill slits are fully formed, the sense organs are completely invaginated, the spinal nerve anlagen are laid down and the limb fundamentals are about to appear. The earliest anlage of the liver is not extensive and can hardly be recognized without a previous study of somewhat later stages. Again after the primitive paired liver diverticula are well formed they are often to a considerable extent obliterated by the falling of the embryo to one or the other side as it becomes top-heavy by separation from the yolk which has supported it up to this time. This flexure causes one or more large transitory folds which tend to render inconspicuous the pouch in the side upon which the embryo comes to lie, and at the same time almost obliterates the opposite pouch by stretching. The histologic characters of these areas remain unchanged however. Figure 13 is a cross section through the liver region of an embryo 4.8 mm. in length, showing these changes.

Although in this form of selachian at least the anlage of the liver is a paired one, it does not follow that this is the original condition of the structure. It seems probable that it has been brought about purely by the mechanical influence of the large

amount of yolk present by which the original tube of entoderm is spread out plate-like upon an almost flat surface of the yolk substance. In such a case the ventral portion of the original tube would form the peripheral portion of each lateral half of the plate and the folding of the plate into a tube again in the course of

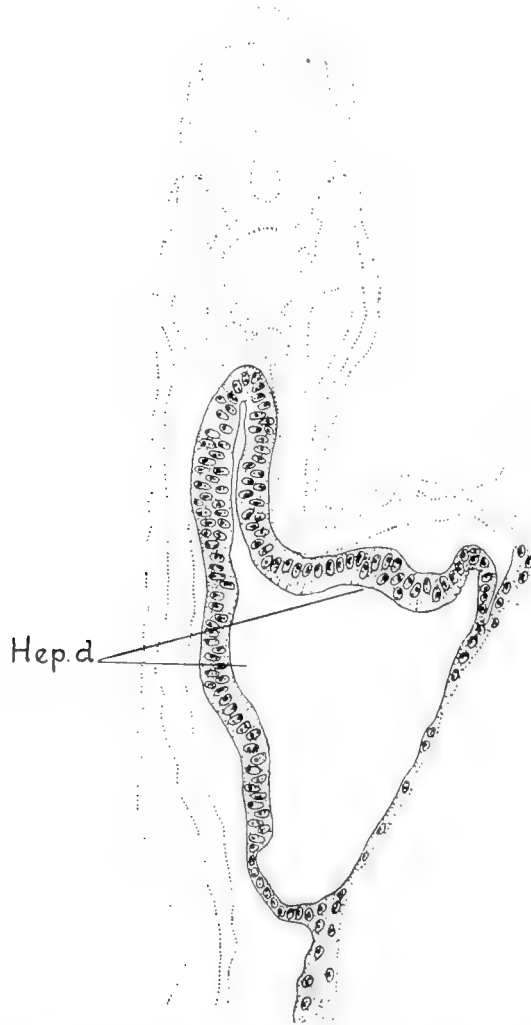


Fig.13 Transverse section of an *Acanthias* embryo 4.8 mm. long (H.E.C. 1398), showing the effect of the inclination of the embryo upon the lateral hepatic diverticula. $\times 50$. *Hep.d.*, hepatic diverticula.

the separation of the embryo from the yolk would approximate once more the two separated parts. These lateral diverticula may then be regarded simply as the potential halves of an original ventral pouch which begin their expansion before their union along the ventral median line takes place. As might be expected

this union does not occur at the same time along the entire antero-posterior length of the liver but proceeds from the cephalic end backward, thus producing the *U*-shaped figure shown in figures 9 and 10.

It appears that too much importance has been placed upon the position of the liver anlagen in regard to the anterior intestinal portal. Laguesse ('93), Brachet ('96) and Choronschitzky ('00) all emphasize this point. This structure, however, is constantly shifting posteriorly as has been demonstrated by Mayr ('97), and the location of the liver in front of it holds good only for stages which are well advanced. The location of the liver as immediately behind the sinus venosus is perhaps of more value, but in *Acanthias* at least, the liver is present before the two omphalomesenteric veins become confluent to form the endothelial heart, or indeed before they are represented by definite endothelial tubes.

Laguesse has already called attention to the fact that in *Acanthias* the gall bladder appears somewhat later than the remainder of the hepatic apparatus, and seems to be developed from the anterior wall of the yolk-stalk rather than from the posterior part of the median liver pouch. Hammar ('93, '97) appears to hold the same opinion in regard to *Torpedo*. I believe that my sections and models bear out this conclusion and that this structure can be properly interpreted as an organ arising quite separate from the hepatic anlage at the juncture of the pars ductus of the lateral liver diverticula and the floor of the gut, as represented by the anterior wall of the yolk-stalk portal. The shifting of the sac anteriorly so that its duct comes to lie in front of the openings of the hepatic ducts into the ductus choledochus will be discussed in the following section.

All specimens after the stage when the median and lateral hepatic pouches are formed show a small but constant rotation of the hepatic anlage to the right. This rotation seems without doubt to be a part of that greater one which produces the spiral valve. Like the latter it is from the left to the right side, i.e., clockwise around an axis corresponding to the longitudinal axis of the gut, and it is coincident with it, appearing when the embryo has from 50 to 60 segments and has reached a length of 6 to 7 mm.

Its extent is but slight as compared with that of the posterior portion of the gut, being at most not over 15 degrees. The hinder and lower portion of the hepatic anlage is less affected by this twisting than is the anterior free part presumably because its attachment to the vitelline duct is still considerable in extent and must offer some resistance to whatever force it may be that produces the rotation. That this portion of the alimentary tract is affected to a slight degree however is shown by the broad shallow groove which appears in the posterior half of the left wall of the yolk-stalk and which has been figured and described in the Normal plates (Scammon '11), under the term 'Lateral groove of the vitelline duct,' and which may be seen in the figures of reconstructions of embryos 7.5, 9 and 11.5 mm. in length respectively, in that paper. The gut anterior to the yolk-stalk also shows some rotation, being twisted to the right as is indicated by the angle formed by its lumen with the mid-sagittal plane of the body.

PART II

I. DESCRIPTION OF FULLY FORMED BILIARY APPARATUS

Before attempting to describe the development of the gall bladder and liver ducts, it may be well to outline the form of these structures in the late embryo or new-born fish and to present the terminology which will be employed in the remainder of this paper.

In large embryos and new-born specimens of *Acanthias* the liver is a large viscus occupying nearly half of the abdominal cavity. It consists of two lateral lobes which are united anteriorly by a median mass which stretches completely across the body cavity posterior to the septum transversum. From the right ventral and posterior margin of the median mass a small pointed process extends backward and to the left. As the gall bladder is imbedded in this mass it has been termed the cystic lobe. The cystic lobe lies directly ventral to the stomach and to the left of the cephalic end of the large internal yolk sac.

The gall bladder is an elongated tubular sac which lies along the right margin of the cystic lobe. It is imbedded in hepatic parenchyma except for a little of the right surface which receives a peritoneal investment. The cystic duct arises from the anterior end of the gall bladder and proceeds directly dorsally. It then

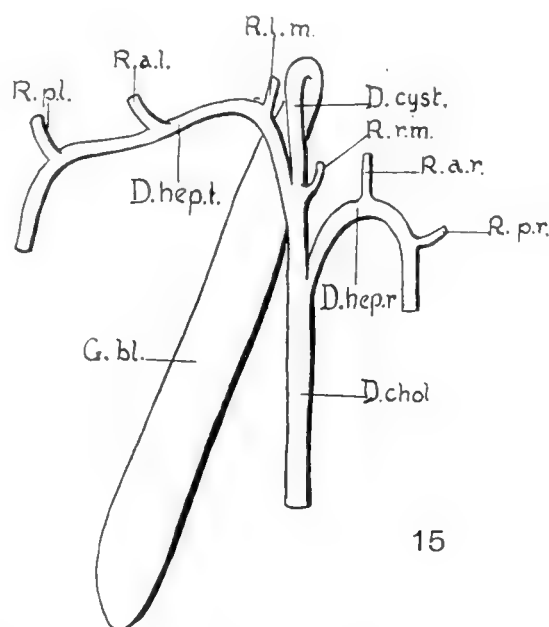
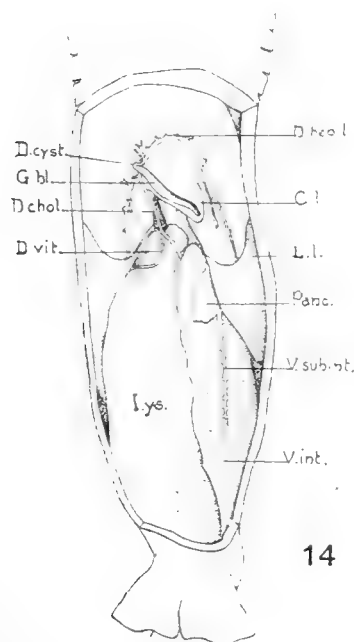


Fig. 14 A dissection of an *Acanthias* embryo 20 cm. in length. $\times \frac{3}{4}$. The ventral abdominal wall has been cut away and the vitelline duct severed at its connection with the internal yolk stalk. The gall bladder and main hepatic ducts have been dissected out. The large veins of the liver have been omitted from this drawing. *C.l.*, cystic lobe; *D.chol.*, ductus choledochus; *D.cyst.*, cystic duct; *D.hept.l.*, left hepatic duct; *D.vit.*, vitelline duct; *G.bl.*, gall bladder; *I.y.s.*, internal yolk sac; *L.l.*, lateral lobe; *Panc.*, pancreas; *V.int.*, valvular intestine; *V. subint.*, subintestinal vein.

Fig. 15 Diagrammatic representation of the gall bladder and liver ducts of *Acanthias* as seen from above. *D.chol.*, ductus choledochus; *D.cyst.*, cystic duct; *D.hept.l.*, left hepatic duct; *D.hept.r.*, right hepatic duct; *G.bl.*, gall bladder; *R.al.*, anterior left hepatic ramus; *R.a.r.*, anterior right hepatic ramus; *R.l.m.*, left medial hepatic ramus; *R.pl.*, posterior left hepatic ramus; *R.p.r.*, posterior right hepatic ramus; *R.r.m.*, right medial hepatic ramus.

makes a sharp semicircular curve and proceeds posteriorly to join the ductus choledochus. In older embryos and adults there is no line of demarcation between the cystic and common bile ducts. The ductus choledochus extends backward ventral to the

internal yolk sac joining the valvular intestine on the left side of the first turn of the spiral valve.

Figure 15 shows the gall bladder and hepatic ducts in diagram. The main right and left hepatic ducts join with the ductus choledochus obliquely, the left gaining entrance in front of the right. The distance between the ostia of the two ducts varies in different specimens. The left duct after extending a short distance anteriorly arches far out laterally and there turning backward passes posteriorly in the left lateral lobe. The right duct makes a sharp arch anteriorly and then passes backward into the right lobe.

The main lateral hepatic ducts give rise to numerous small hepatic tubules and to several larger rami. The former are extremely irregular in form, origin and number, but the latter, although displaying great variation in position can in most cases be reduced to the following classification: (1) right medial hepatic ramus, (2) left medial hepatic ramus, (3) anterior right hepatic ramus, (4) posterior right dorsal hepatic ramus, (5) anterior left hepatic ramus, (6) posterior left dorsal hepatic ramus.

The right medial hepatic ramus varies considerably in the place of origin, commonly it is attached to the right duct near its proximal end. The left medial hepatic ramus commonly takes origin from the proximal part of the left hepatic duct but may in some cases be attached to the ductus choledochus or even to the base of the right hepatic duct. The anterior right and the anterior left rami generally arise from the summit of the anterior arch formed by each of the main hepatic ducts, but the left ramus may attach either to the ductus choledochus, or as I have observed in one embryo, to the base of the main right hepatic duct. The posterior dorsal hepatic rami arise from the hepatic ducts either at the lateral extremity of the anterior arch or in the anterior part of their posterior course. They seem to be fairly constant in position. Minor variants from the above scheme are common and simple rami differ much in size or may be replaced by two or more smaller ones. The terminology used here is based upon the development of these structures as will now be described.

II. DEVELOPMENT OF THE HEPATIC DUCTS AND THEIR RAMI

The elements entering into the formation of the hepatic ducts are the anterior portion of the median liver pouch or *pars hepatica mediana*, and the right and left lateral hepatic pouches or the *pars hepatica lateralis*.⁴ These structures are converted into the main hepatic ducts found in the fully developed embryo by means of reduction in caliber both relative and actual, by elongation, and by partial separation from the posterior portion of the median liver pouch or *pars ductus mediana*. That such processes take place has been recognized by Balfour ('76), Hammar ('93), Brachet ('96), and other investigators. The details have not been described. The minor ducts are formed in *Acanthias* in much the same manner as are the major ones, by the differentiation and elongation of the proximal parts or pedicles of certain fairly definitely placed groups of tubules which arise from the surface of the embryonic structures which form the main ducts. This method of formation of the minor ducts probably holds only for selachians in which the omphalo-mesenteric veins are comparatively small and develop at a late stage.

An account of the development of these ducts may begin with the description of an embryo 15 mm. in length (H.E.C. 227 and No. 26 of the Normal plate series) the general anatomy of which is illustrated in figure 13 of the Normal plates of *Squalus acanthias*.

The main divisions of the liver of this specimen and the proximal part of the hepatic tubules arising from them have been reconstructed and figures 41, 42 and 44 are right lateral, left lateral and anterior views of this object.

The median hepatic pouch is completely separated from the gut above and this separation has extended backward convert-

⁴ The use of this and the following terms of this paragraph is somewhat of a departure from the classification of the components of the selachian liver pouch into '*pars cystica*' and '*pars hepatica*' as proposed by Brachet ('96, '97) on the basis of a similar classification employed by Goeppert ('93) in his description of the development of the liver in Teleosts. The term '*pars cystica*' as used by Brachet includes the '*pars ductus mediana*' and '*pars ductus lateralis*' as employed here, as well as the anlage of the gall-bladder and cystic duct. If the conception as presented here, of the gall bladder as an organ with an origin distinct from the liver, is a correct one, then this term '*pars cystica*' can hardly be properly applied

ing the pars ductus lateralis into a short tube of large caliber. This is the middle part of the ductus choledochus. It joins with the floor of the duodenum a little to the right, thus preserving the same relation observed in younger embryos. The obliquely placed swelling upon the dorsal surface of the median pouch which represents the course of the distal portion of the ductus choledochus is present but is not so marked as in the embryo 10 mm. in length described in the preceding section (p. 348).

The pars hepatica mediana or anterior part of the median pouch is broadly continuous with the pars ductus medialis behind and with the lateral pouches posteriorly and laterally. Its anterior surface (fig. 44) is rendered extremely irregular by the formation of a number of hepatic tubules. The origin of these structures from ridges in the pars hepatica was noted in connection with the description of an embryo 10 mm. in length, in the preceding section of this paper. At the present stage the tubules arising from the pars hepatica mediana are little more than short conical evaginations of the pouch wall and only one shows any evidence of the complex branching which all soon undergo. The tubules of the pars hepatica mediana are divided into two groups, a right and a left, by a deep vertical furrow which lies somewhat to the left of the median plane and extends from the dorsal to the ventral surface of the pouch. The right subdivision thus formed is more extensive than the left, but a smaller number of tubules arise from it. The groups of tubules established by this subdivision will be termed in this paper the right medial group and the left medial group respectively. The lower surface of the pars mediana remains smooth at this stage and rests upon a mass of mesenchyma which extends from the anterior surface of the gall bladder to the anterior mesothelial wall of the liver.

to all of these structures and to use it for structures which are later wholly incorporated in the ductus choledochus and not in the vessica fellae or its duct at all, seems inadvisable. The use of the expression 'mediana' in connection with 'pars ductus' is not intended to convey the meaning that this portion of the ductus choledochus is a direct derivative of the median part of the gut primarily, but that it is formed from the median pouch produced by the fusion of the anterior parts of the original lateral diverticula while the more posterior part of the ductus choledochus is formed from the hinder parts of the lateral diverticula without the intervention of a median pouch stage.

The lateral pouches merge into the pars hepatica mediana anteriorly and the distinction between these parts in this region is only possible through an examination of the tubule formation. Posteriorly, however, the proximal portion or stalk of each pouch is constricted and elongated to form the hinder part of a broad short duct connecting the pars ductus of the median pouch with the distal expanded portions of the lateral ones. This condition is illustrated by the transverse section shown in figure 16.

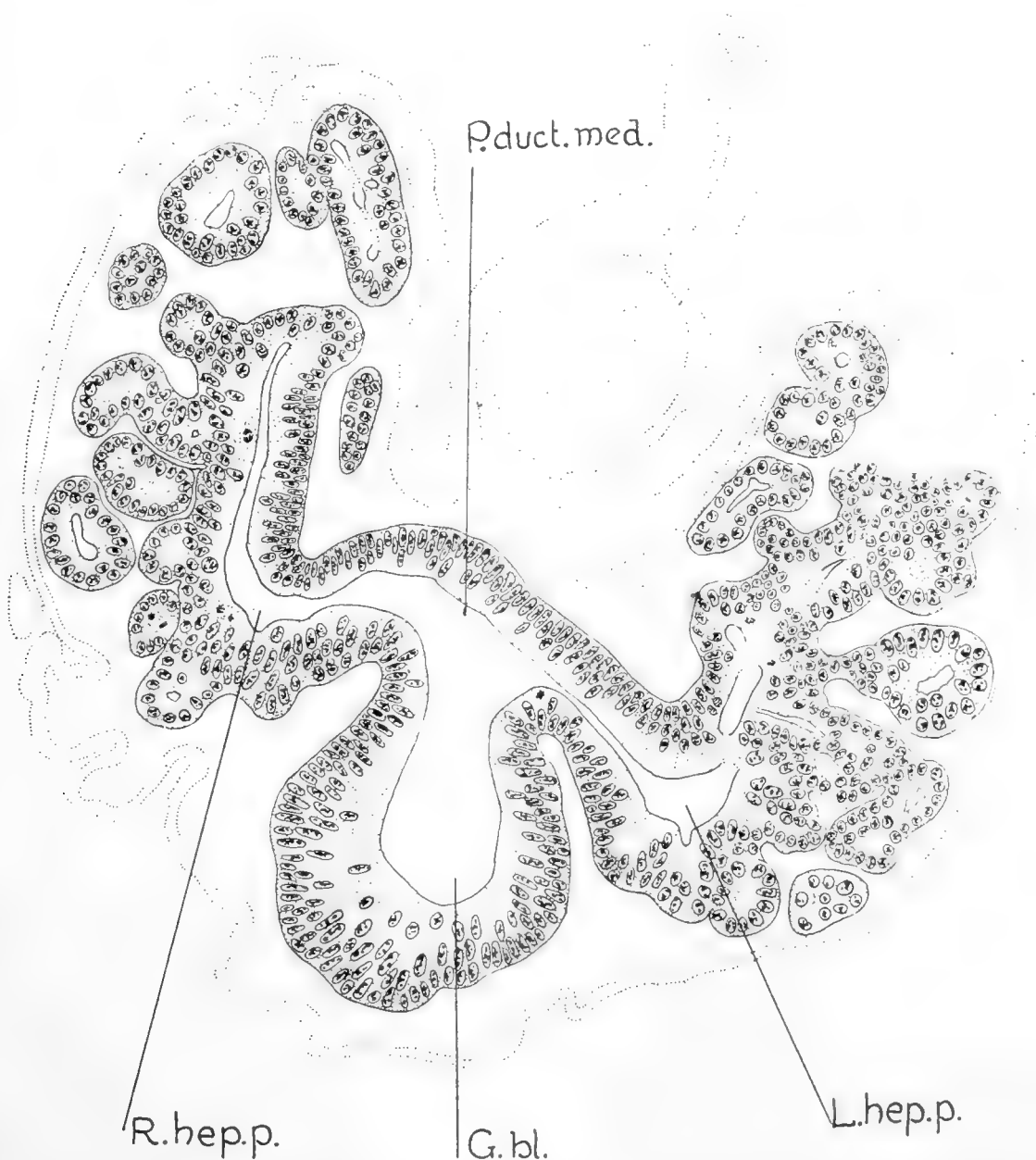


Fig. 16 Transverse section through the liver of an *Acanthias* embryo 15 mm. long (H.E.C. 227). $\times 125$. *G.bl.*, gall bladder; *L.hep.p.*, left hepatic pouch. *P.duct.med.*, pars ductus mediana; *R.hep.p.*, right hepatic pouch.

The mesial surfaces of the lateral pouches are smooth except for some minor fissures and the gutter like spaces between them and the pars mediana are occupied by mesenchyma and the vitelline veins. The lateral surface of each pouch is almost obscured by the numerous hepatic tubules which arise from it. The dorsal growth of the pouches so evident in early stages has now come to an end and their dorsal margins hardly extend above the ventral surface of the stomach as is seen in figures 41 and 42. A number of large trunk-tubules arise from them. As in the pars hepatica mediana all the tubules of the lateral pouches tend to gather in certain fairly well defined groups. These consist, on either side, of an anterior and a posterior group, and the latter is less definitely subdivided into a dorsal and a ventral cluster. The tubules of the anterior groups spring from the dorsal half of the anterior part of the lateral pouch leaving a ventral area below which is smooth or occupied only by small tubules in the process of formation. The posterior group is much larger and its two subdivisions occupy the entire posterior half and hinder margin of the pouch. As will be seen from figure 41, these groups are not completely separated, as small and less developed tubules intervene in some places. The formation of these minor tubules continues until a much later period. The tubule groups of the anterior part of the left lateral pouch and of the left side of the pars hepatica mediana lie much closer together than those of the opposite side.

This early arrangement of the hepatic tubules into groups is of much importance for, as has been stated, while the main hepatic ducts are produced by the elongation and narrowing of the caliber of the lateral pouches, each group of tubules becomes isolated by the formation of a common stalk which later develops into one or more rami of the minor hepatic ducts. The arrangement of the tubule groups is expressed in tabular form in table 1.

A reconstruction, illustrated in figures 43, 45 and 46 of an embryo but 0.5 mm. longer than the preceding but which resembles in general anatomy the average embryo of 18 mm., shows more clearly the process of duct formation. The common bile duct is now three times as long as its greatest diameter and the pars duc-

tus mediana has lost somewhat of its sac-like form and appears as an irregular dilated chamber which is broader in front than behind and receives the broad short cystic duct from below and the proximal parts of the lateral pouches from the sides. The pars hepatica mediana is somewhat elongated and is still broadly continuous with the pars ductus posteriorly. The formation of tubules from it has progressed considerably and now involves the ventral as well as the anterior surface. The division of this part of the median pouch into right and left segments by a vertical fissure is well marked. This fissure lies in the same sagittal plane as the left lateral wall of the pars ductus behind it, thus showing a marked

TABLE 1

Arrangement of hepatic tubule groups

Pars hepatica mediana					
Right medial tubule group			Left medial tubule group		
Right hepatic pouch			Left hepatic pouch		
Anterior right tubule group	Posterior right tubule group		Anterior left tubule group	Posterior left tubule group	
	Dorsal cluster	Ventral cluster		Dorsal cluster	Ventral cluster

shift to the left. On the right side the pars hepatica mediana is no longer confluent with the anterior part of the lateral pouch but is separated from it by narrow zone of tubule free surface (fig. 46). On the left side however the pars hepatica mediana extends far laterally and is continuous with the left lateral pouch posteriorly.

The lateral pouches also show several changes. Their proximal stalks are elongated and the size of their distal expansions is much reduced. The connecting stalk of the left pouch with the pars ductus mediana is shifted so far forward that its posterior margin lies in the same transverse plane as the anterior margin of its fellow of the opposite side. The distal part of the left pouch is

also farther separated from the posterior part of the median pouch than is the right.

The grouping of the tubules which arise from the lateral pouches is quite distinct except for the left medial and anterior left groups which have been rendered confluent by the vascular changes just discussed. The dorsal and ventral posterior clusters of the left side (fig. 45) are separate and the dorsal cluster which throughout early stages precedes the ventral one in development, is raised from the pouch surface and connected with it by a short broad pedicle. On the right side (fig. 46), the anterior right tubule group lies close to but is not fused with the right medial one. As on the opposite side, both dorsal and ventral clusters are distinct, and both are beginning to develop pedicles.

These differences between the right and left parts of the *pars hepatica mediana* and between the lateral pouches were present to some small degree in the preceding stage, but are more noticeable in this specimen and become more marked during later development. They are due primarily to the unequal size of the omphalo-mesenteric veins.

It is well known from the studies of Rabl ('92), Mayer ('89), Hammar ('93), and others, that in selachians there are at first two omphalo-mesenteric veins of almost equal size. Early in development however the right omphalo-mesenteric vein loses its connection with the *area vasculosa* and for a time ends blindly on the lateral wall of the yolk-stalk. Later a connection is formed between the posterior end of the right omphalo-mesenteric vein and the subintestinal vein by means of a channel lying to the right of the pancreas. During the period while the right omphalo-mesenteric vein ends blindly behind the blood from the yolk sac and from the subintestinal vein passes forward through the left omphalo-mesenteric vein alone, and this vessel in consequence becomes much enlarged. At this time the rotation of the gut from left to right about a longitudinal axis is in progress and the passageway for the vascular channel between the lateral hepatic pouch and the median hepatic pouch and fore gut is somewhat larger on the left side than on the right. This condition is shown both in the anterior view of the model of an embryo

10 mm. long and in figure 8 A, a cross section of the same specimen.

The effect of the enlargement of the left omphalo-mesenteric vein upon the liver anlage has already been described in part and will be considered farther in describing later stages. Passing along the left side of the median hepatic pouch or the common bile duct it pushes this structure to the right and shifts the left

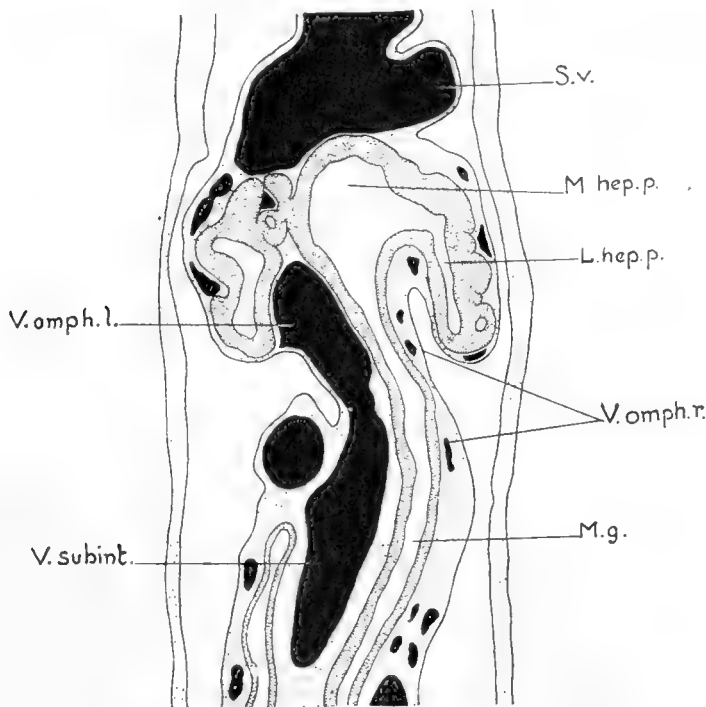


Fig. 17 Frontal section of the liver and mid gut region of an *Acanthias* embryo 13 mm. long (H.E.C. 226). $\times 40$. *L.hep.p.*, lateral hepatic pouch; *M.g.*, mid gut; *M.hep.p.*, median hepatic pouch; *S.v.*, sinus venosus; *V.omph.l.*, left omphalo-mesenteric vein; *V.omph.r.*, right omphalo-mesenteric vein; *V.subint.*, subintestinal vein.

hepatic pouch or left hepatic duct anteriorly and laterally. This process is shown in an early stage by figure 17. At the same time the vessel upon encountering the left segment of the anterior and expanded part of the median hepatic pouch breaks up into several trunks which pass below and above the obstruction. One of the larger trunks passes through the dorsal part of the vertical cleft between the right and left anterior tubule groups or hepatic

rami and gradually enlarging this space presses the tubules apart until it forms one of the larger channels of the vein.

The right omphalo-mesenteric vein, after establishing its posterior connection with the subintestinal vein, which it does when the embryo reaches a length of about 10 mm., grows rapidly, although it never equals in size that of the opposite side, and does

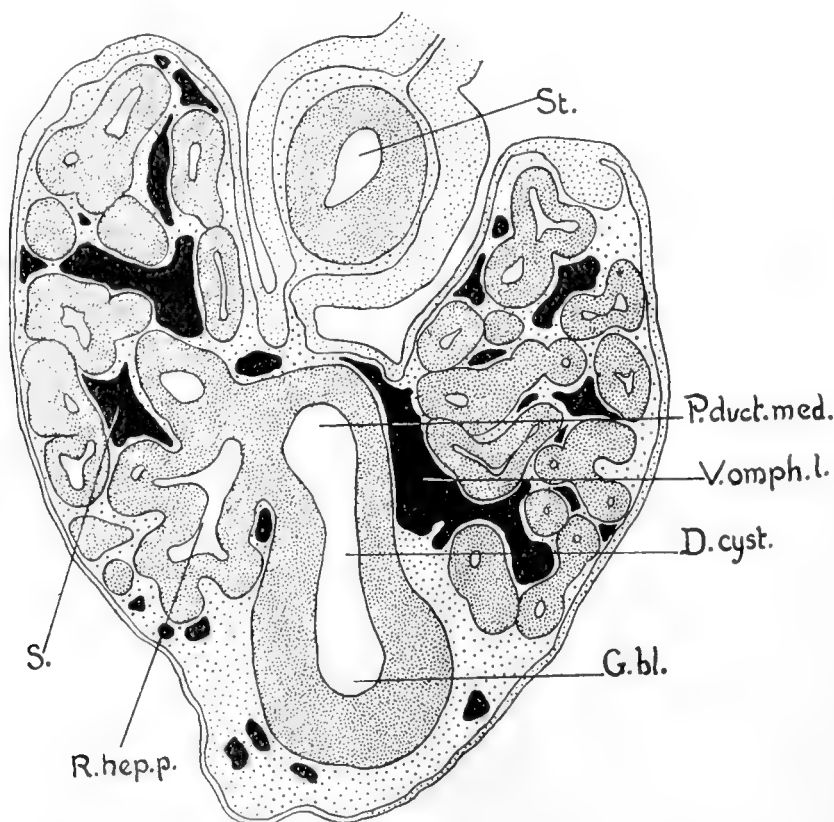


Fig. 18 Transverse section of an *Acanthias* embryo, 15.5 mm. long (S.C. 1). $\times 100$. *D.cyst.*, cystic duct. *G.bl.*, gall bladder; *P.duct.med.*, pars ductus mediana; *R.hep.p.*, right hepatic pouch; *S.*, sinusoids of right omphalo-mesenteric vein; *St.*, stomach; *V.omph.l.*, left omphalo-mesenteric vein.

not affect the position of the biliary apparatus to any great degree. At first the right and left omphalo-mesenteric veins pass forward to meet in front of the anterior portion of the median hepatic pouch but in later stages the vessels become confluent behind and below the anterior part of the median hepatic pouch or its derivative, thus forming a large sinus which increases the effect already begun by the left vitelline vein, viz., shifting the common

bile duct and cystic duct to the right and the left hepatic forward and laterally. This condition is shown by a frontal section of a much older embryo in figure 19.

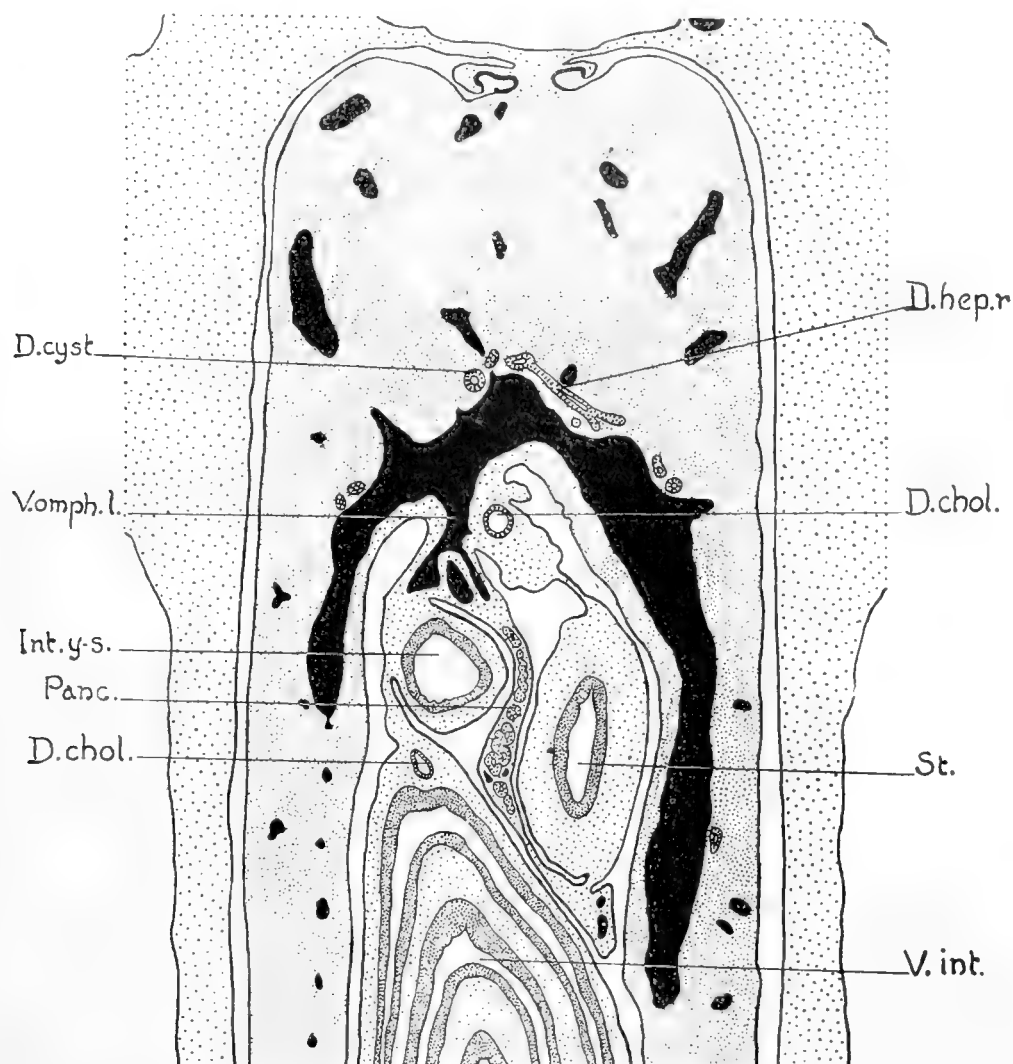


Fig. 19 Frontal section of an *Acanthias* embryo 41 mm. long (H.E.C. 371). $\times 40$. *D.chol.*, ductus choledochus; *D.cyst.*, cystic duct; *D.hep.r.*, right hepatic duct; *Int.y.s.*, internal yolk sac; *Panc.*, pancreas; *St.*, stomach; *V.int.*, valvular intestine; *V.omph.l.*, left omphalo-mesenteric vein.

An embryo 20.5 mm. in length shows sufficient differentiation of the embryonic hepatic structures to permit the introduction of the adult terminology in describing them. As will be seen from a cross section through the anterior end of the gall bladder of a specimen of nearly the same stage (fig. 20), both lateral and median pouches are reduced in diameter and the tubule groups, in

part at any rate, are connected with the latter by short ducts. A reconstruction of the biliary apparatus of this stage is shown in figures 47, 48 and 49.

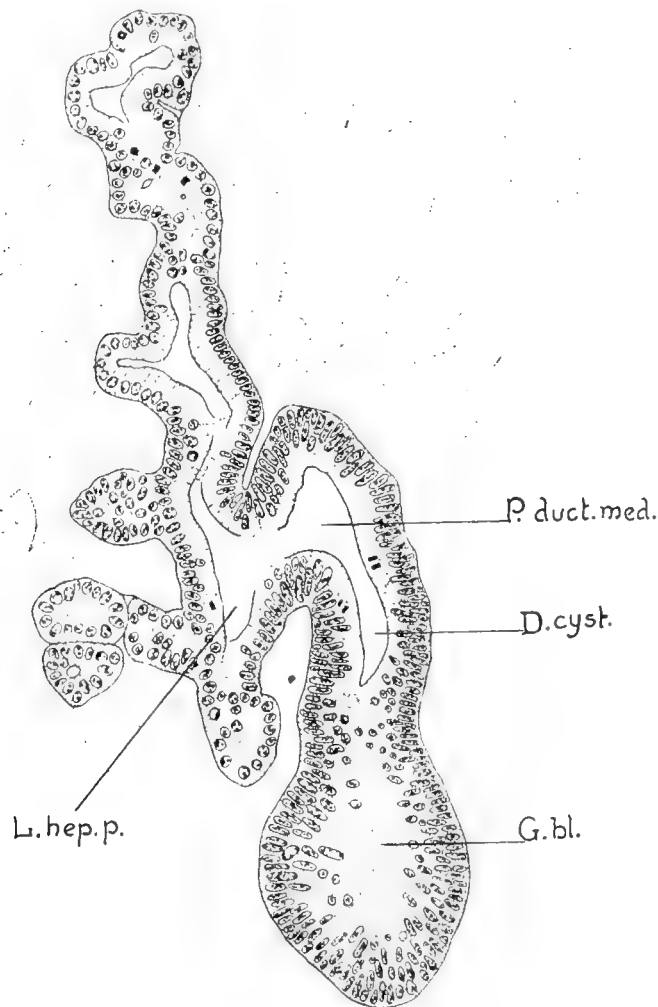


Fig. 20 Transverse section of an *Acanthias* embryo 19 mm. long (S.C. 2). $\times 100$. *D.cyst.*, cystic duct; *G.bl.*, gall bladder; *L.hep.p.*, lateral hepatic pouch; *P.duct.med.*, pars ductus mediana.

The pars ductus mediana, or as the structure may now be termed, the terminal part of the ductus choledochus, is tubular in form. The pars hepatica mediana is reduced in size antero-posteriorly and expanded laterally. The vertical cleft dividing it into right and left parts lies lateral to the left wall of pars duc-

tus and is accentuated by the two short ducts which arise on either side of it. These ducts are the right and left medial hepatic rami and are the derivatives of the right medial and left medial tubule groups respectively. As yet they are very short and large calibered and break up almost immediately into a number of hepatic tubules. Two tubules arising immediately ventral to the left medial ramus probably represent the remains of the anterior left tubule group whose fusion with the left medial group has been described. Three additional tubules of large caliber arise from the anterior and ventral surface of the *pars hepatica mediana*. They may be derived from the right or left medial group but probably have arisen direct from the pouch wall after the main tubule groups were established.

The left hepatic pouch or duct as we may now term the structure, takes origin entirely from the lateral part of the posterior surface of the *pars hepatica mediana*, having been entirely separated from the *pars ductus* mesially. Thus it is already evident that the adult hepatic duct upon this side is made up of two elements, a proximal and transverse part derived from the left part of the *pars hepatica mediana* and a distal and longitudinal part formed from the left lateral pouch proper. On the opposite side the pouch or duct arises, as in preceding stages, from the lateral surface of the *pars ductus* or common bile duct. A distinct groove separates the anterior boundary of the right duct from the *pars hepatica mediana*. Also as in earlier stages the left duct is widely separated from the *pars ductus* while the right duct lies quite close to its opposite side. The condition of the anterior left tubule group has already been described and on the right side the anterior group is connected with the main hepatic duct by a distinct neck, the anterior right hepatic ramus, which is directed dorsally. On the left side the dorsal posterior cluster or ramus is no farther developed than before, but the ventral cluster possesses a short duct which extends posteriorly and bifurcates into upper and lower branches. On the right side the dorsal posterior cluster is represented by two ducts, the upper one being particularly prominent and directed posteriorly. The posterior ventral cluster arises from a very short broad diverticulum of the

pouch and breaks up into five large tubules, the largest of which is directed posteriorly.

In an embryo of 20.6 mm. (H.E.C. 1494, No. 28, Normal plate series) the hepatic ducts are so completely formed that their origin from pouches and tubule groups would hardly be surmised from the reconstruction of them seen in figure 50. The pars ductus mediana is not separable from the more posterior part of the ductus choledochus and the left part of the pars hepatica mediana and the left lateral pouch form together one duct, the left hepatic, which arises from the lateral surface of the ductus choledochus and curves first laterally and then backward. The distinct angle between the transverse and longitudinal parts of the duct is the point of union of the two elements which form it. The right medial hepatic ramus arises at the union of the left hepatic duct with the ductus choledochus. Immediately to the left of this is a smooth narrow segment of the left hepatic duct which lies between two branches of the left vitelline vein. To the left of this segment lies a dorsal duct, the left medial ramus, and below and lateral to it is the anterior left ramus. These represent the anterior left and left medial tubule clusters respectively. The small tubules which are seen in the anterior view of the model arising between these two ducts are probably derived in part from both tubule groups which, it will be remembered, were somewhat fused in earlier stages.

The right hepatic duct is much shorter than the left. It takes origin from the anterior end of the ductus choledochus and curves backward at once. A very large dorsal ramus arises from its upper surface just distal to its connection with the ductus choledochus. This is the anterior right hepatic ramus and corresponds to the tubule group of the same name in younger embryos. Aside from this and one small tubule arising from the anterior surface, the proximal part of the right hepatic duct is smooth. Near its distal end it gives off several minor tubules. The dorsal ones represent the dorsal posterior tubule cluster while the ventral ones are derived from the ventral posterior tubule cluster as is also the most distal part of the duct itself. By this stage all the minor hepatic rami are established and no new tubules arise from

the ducts direct, all further increase in hepatic parenchyma being due to growth in the hepatic trabeculae proper.

The later histories of the major and minor hepatic ducts can be most easily undertaken separately. Up to the stage just described the gradual shifting of the anterior end of the ductus choledochus to the right has been a constant feature. This process becomes more noticeable with the distinct differentiation of the hepatic ducts and by the time the embryo reaches a length of from 25 to 28 mm., the anterior end of the duct may be so rotated that the original anterior surface faces the right and the left surface appears as the anterior one. The rotation though always present is not always so extreme as this type which is shown in figures 51 and 52. This process is brought about by a distinct enlargement of the sinus formed by the omphalo-mesenteric veins which lies below and to the right of the ductus choledochus. The rapid growth of this sinus is in turn due to changes in the hepatic trabeculae. Until the embryo reaches the length of from 25 to 28 mm. the trabeculae form a wide meshed network and the blood is able to flow around them through sinusoids of large caliber. From this stage on however the trabeculae increase rapidly in size and the surrounding sinusoids are reduced to extremely small vascular channels.⁵ The blood is thus directed in a large part into the omphalo-mesenteric veins and their caliber is considerably increased.

The main hepatic ducts are markedly influenced by this rotation. The left one is carried forward until almost its entire length lies in the transverse plane of the embryo and only the distal end with the tubules arising from it project backward while the right hepatic duct extends directly backward with little or no lateral course (figs. 21, 51, 52 and 54). Soon after the rotation of the anterior end of the ductus choledochus takes place the cystic duct, which until this time has joined with the ductus choledochus from below, is carried forward and upward along with the gall bladder until it joins with the anterior surface of the ductus choledochus and appears at first sight as an anterior extension of

⁵ This fact has already been recognized and recorded by Minot ('00).

that structure. This process, illustrated in figures 22, 23, 24, 25 and 54, is brought about in part at least by the growth of the internal yolk sac and is discussed in more detail in the section upon the development of the gall bladder. A distinct forward arch appears in the proximal portion of the main hepatic ducts so that instead of entering the ductus choledochus at right angles to its longitudinal axis as in younger stage, they extend backward on either side of it for a short distance and then join with it very obliquely. This arch may be partially brought about by the hepatic ducts being forced forward by the same agency as that influencing the cystic apparatus at this time but probably it is also due in part to the actual shifting backward of the ostia of the hepatic ducts along the ductus choledochus.

Up to the stage represented by the embryo of 20.6 mm., the hepatic ducts were described with little difficulty for they pursue much the same course in all the specimens which were examined. However, the reduction in size of the distal end of the ductus choledochus and the shiftings which it and the hepatic ducts undergo modify considerably the position of some of the minor ducts and to varying degrees in different specimens. This applies particularly to the anterior left and right lateral rami and the left and right medial rami. The posterior dorsal rami remain fairly constant in position at the juncture of the transverse and posterior parts of their respective trunks, and the posterior ventral rami, while giving rise to new short minor sprouts in their course, seem fairly regular.

Of the several types observed in embryos and specimens of the pup stage, the commonest and simplest one is that in which rotation of the ductus choledochus is present but not extreme and in which the embryonic arrangement of the rami is to a large extent retained. This form is illustrated by the graphic reconstruction of two embryos, one 28 mm. and one 41 mm. in length illustrated in figures 21 and 22.

The variants from this type so far as observed have been such as might be expected from farther rotation of the anterior part of the ductus choledochus. If this process is extreme all those rami which arise from the anterior surface of the pars hepatica

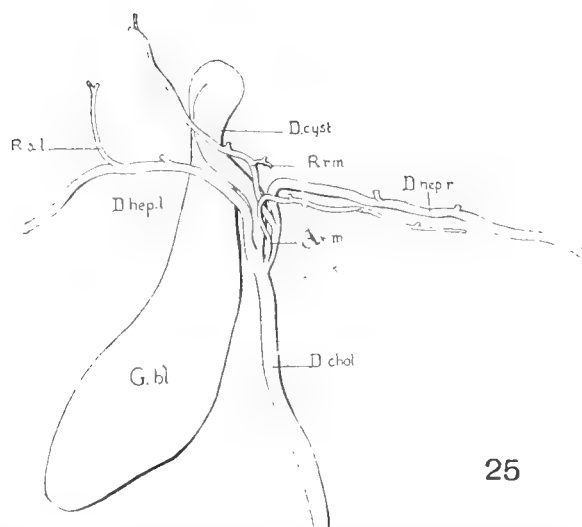
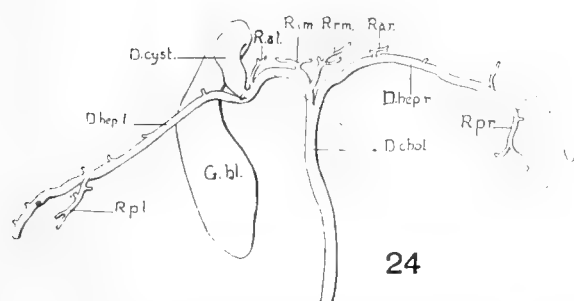
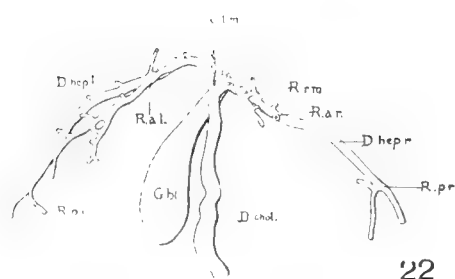
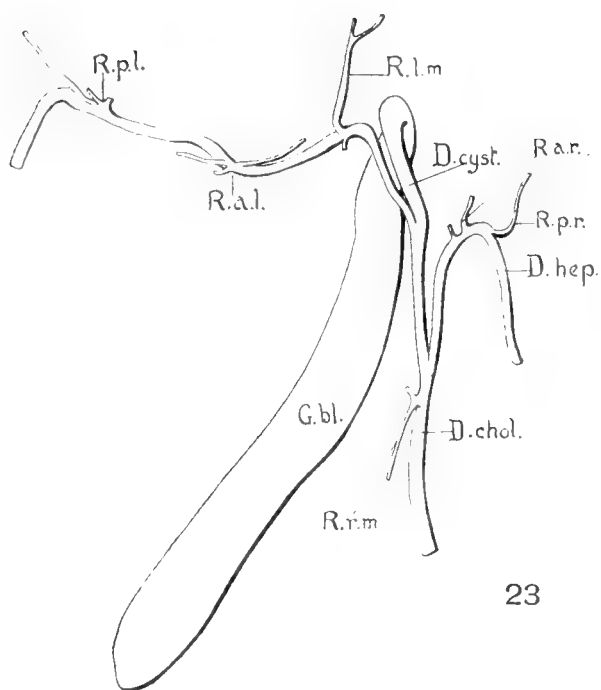
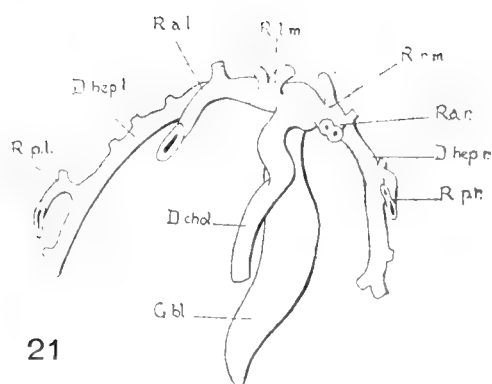


Fig. 21 Graphic reconstruction (dorsal view) of the gall bladder and liver ducts of an embryo 28 mm. long (H.E.C. 221). $\times 20$. For abbreviations, see figure 22.

Fig. 22 Graphic reconstruction (dorsal view) of the gall bladder and liver ducts of an embryo 41 mm. long (H.E.C. 371). $\times 20$. *D.chol.*, ductus choledochus; *D.cyst.*, cystic duct; *D.hep.l.*, left hepatic duct; *D.hep.r.*, right hepatic duct; *G.bl.*, gall bladder; *R.al.*, anterior left hepatic ramus; *R.ar.*, anterior right hepatic ramus; *R.l.m.*, left medial hepatic ramus; *R.r.m.*, right medial hepatic ramus; *R.pl.*, posterior left hepatic ramus; *R.p.r.*, posterior right hepatic ramus.

Fig. 23 Graphic reconstruction (dorsal view) of the gall bladder and liver ducts of an embryo 18 cm. in length. $\times 10$. For abbreviations, see figure 22.

Fig. 24 Graphic reconstruction (dorsal view) of the gall bladder and liver ducts of an embryo 60 mm. in length (H.E.C. 409). $\times 20$. For abbreviations, see figure 22.

Fig. 25 Graphic reconstruction (dorsal view) of the gall bladder and liver ducts of an embryo 86 mm. in length (H.E.C. 410). $\times 20$. Only the proximal part of the hepatic ducts are represented. For abbreviations, see figure 22.

mediana and apparently even the anterior left ramus may be carried dextrally until they face the right. Apparently also any intermediate step between this extreme and the embryonic type described above may exist. When rotation first takes place and before the cystic duct begins to shift upward and forward the ostia of the minor anterior ducts remain quite close to that of the main left hepatic duct. With this change however the curve formed by the extreme anterior part of the ductus choledochus and the cystic duct is gradually obliterated so that the two ducts come to form together a slender tube which extends antero-posteriorly in frontal plane, and the ostia of the rami which formerly were on the anterior surface of this curve come to lie on one side or the other of duct and are separated by its dorsal surface. Figures 51 and 52 are of a reconstruction of the biliary apparatus at a stage immediately after a distinct dextral rotation of the ductus choledochus has taken place and before the dorso-anterior migration of the cystic duct and gall bladder is very noticeable. Had this specimen continued its growth, if one may judge from reconstructions of later embryos, the cystic duct would probably be carried forward and upwards in such a way that it would intervene between the left medial ramus and the left main hepatic duct in such a way that it would receive the opening of the former on the right hand side and the latter on the left. Figures 53 and 54 of an embryo 33.1 mm. in length shows a somewhat later stage in which the cystic duct is being forced forward and the right medial ramus which formerly lay almost in the median line is being carried over to the right side of the duct.

Some reconstructions which illustrate the results brought about by the above processes may be illustrated here as they also show the changes which take place in the major ducts and gall bladder in the later periods of embryonic development.

Figure 23 of a very late embryo 18 cm. in length shows a variant in which the left medial ramus has remained attached to the main left hepatic duct but in which the right medial ramus arises from the ductus choledochus. Except for this change, the rami have remained in their early embryonic position. Figure

24 of an embryo 60 mm. in length shows a specimen in which the rotation of the ductus choledochus to the right must have been very great indeed for it still shows an almost right angled curve in that direction at this late stage and the right medial, the left medial, and the anterior left rami all arise from it between the ostia of the cystic and left hepatic and the right hepatic duct. The figure of an embryo of 86 mm. (fig. 25) shows the most extreme case of the series in which both the left and the right anterior rami arise from the right hepatic duct, the latter almost at its ostium. I can only explain this case on the supposition that the posteriorly directed proximal segment of each the right and left main hepatic ducts is derived in part by a splitting off from the ductus choledochus and that the left medial ramus originally connected with an area of the right side of the ductus choledochus which was later incorporated in the distal end of the right lateral hepatic duct.

That other variations in the arrangement of these hepatic rami may occur both in embryo and adult is very probable, but it is to be expected that they will all be of the same general type as those described above, viz., such as may be brought about by the progression of their ostia from left to right. Besides the variation in position of ostia, there are also noticeable ones in the size of the various elements. This seems to be compensatory in that the reduction in extent of any element is accompanied by an increase in size and complexity of its neighbors. Occasionally one element may be replaced by two or more smaller ones and minor rami are commonly found irregularly placed in regard to the larger ones. These minor sprouts as already stated arise either from tubules intermediate in position to the main tubule groups or from such tubules as arise after these groups are somewhat separated from the main ducts by pedicles. A scheme of the origin of the minor ducts or rami will be found in the general summary in table 3.

III. DEVELOPMENT OF THE GALL BLADDER AND CYSTIC DUCT

The gall bladder has been described in the first section of this paper as arising as a median outpouching of the anterior wall of the yolk-stalk immediately below the point where that structure becomes continuous with the floor of the fore gut. The ventral zone of the lateral walls and the floor of this part of the gut at this early stage are a part of the hepatic area and that part of the anterior wall of the yolk-stalk from which the gall bladder arises is later incorporated in the gut behind the liver as the yolk-stalk becomes constricted antero-posteriorly. The time of appearance of the anlage of the gall bladder is somewhat later than that of the liver as has been noted by Laguesse ('93) and Debeyra ('09). It seems possible, from these observations, to regard the gall bladder in this form as derived from a secondary pouch which is formed in the floor of the archenteron immediately behind the liver pouch proper. If this interpretation be correct, an anterior shifting of the gall bladder by which its opening reaches its later position at the anterior end of the ductus choledochus in front of the openings of the hepatic ducts is to be expected. Evidences of such a shifting are not wanting. The early demarkation of the gall bladder has already been described in the preceding section, and has been seen to consist of the formation of dorsal longitudinal grooves and a posterior and ventral vertical one by which the gall bladder is cut off from the anterior wall of the yolk-stalk behind and remains attached to the liver pouch above by a transversely constricted neck which extends along its entire dorsal surface. The anterior end of this neck is less constricted and represents the cystic duct. These changes are illustrated in figures 33, 35 and 37, and are also shown in *B* of figure 26 below.

These changes are even more marked in an embryo of 10 mm. illustrated in figures 39 and 40, and in *C* of figure 26. Along with them is a considerable lateral expansion of the gall bladder and a curious growth of its anterior wall which produces a pointed process which projects forward below the ventral wall of the median hepatic pouch. The gall bladder in this and the preceding stages which follow its separation from the anterior wall of the

yolk-stalk behind lies a little to the left of the median line. This asymmetric position has already been discussed in Part I. It is associated with the rotation of the gut in connection with the formation of the spiral valve.

When the liver reaches the stage represented in figures 41 and 42, of an embryo of 15 mm., the gall bladder is so far separated from it as to form a thick walled ovoid sac, the posterior half of which is rounded and free and the anterior end drawn out into a cone shaped projection. It is still broadly attached

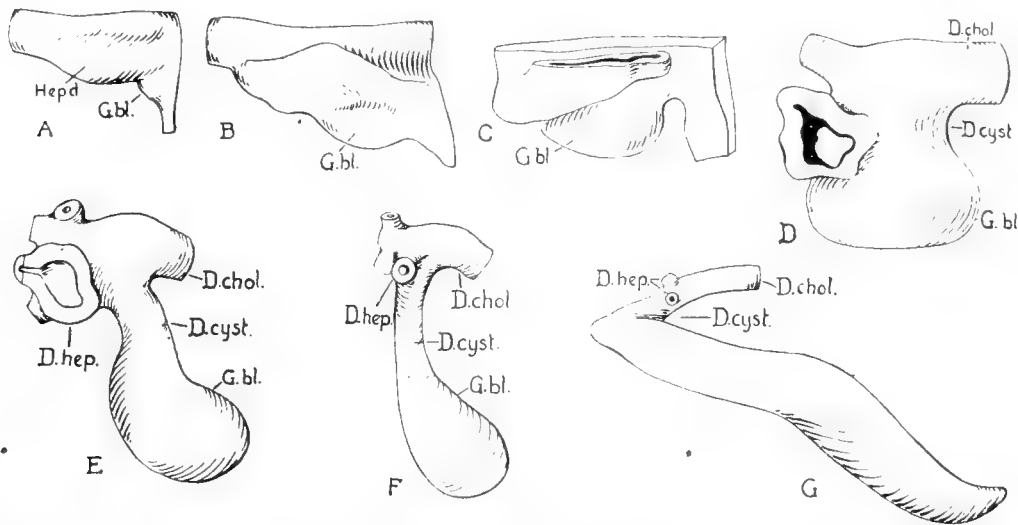


Fig. 26 A series of semi-schematic figures of the development of the gall bladder and cystic duct. A, embryo of 7 mm.; B, embryo of 7.5 mm.; C, embryo of 10 mm.; D, Embryo of 15.5 mm.; E, embryo of 19 mm.; F, embryo of 20.6 mm.; G, embryo of 33.1 mm. All from plastic reconstructions with the exception of A, which is based upon longi-sections. *D.chol.*, ductus choledochus; *D.cyst.*, cystic duct; *D.hep.*, hepatic duct; *G.bl.*, gall bladder; *Hep.d.*, hepatic diverticulum.

to the median liver pouch above. The posterior end extends downward so that the long axis of the sac is no longer parallel with that of the median liver pouch above it.

The gall bladder in an embryo of 15.5 mm. (figs. 45 and 46 and D, fig. 26) is smaller relatively, and the connection between it and the liver above is drawn out into a short broad stalk, the cystic duct. The pointed anterior extremity is less noticeable than in the two preceding specimens and from this time on no evidences are seen of it. In this specimen, also, the gall bladder

lies to the left of the median line and the cystic duct is inclined to the right as well as upward to meet the median hepatic pouch or ductus choledochus.

At 19 mm., the gall bladder is widely separated from the ductus choledochus and inclined at a distinct angle to it. The cystic duct is decidedly elongated and extends upward to the right and decidedly forward to meet the ductus choledochus. This elongation is due in part to the increase in size of the left omphalomesenteric vein, which lies in this region between the gall bladder below and the ductus choledochus above, and perhaps also in part to the great growth of the hepatic trabeculae which takes place at this time. Probably the increased length of the duct is derived in part at least from the lower part of the ductus choledochus from which the anterior end of the cystic duct seems to be separating. This separation is peculiar in that it takes place mesially more rapidly than laterally, thus forming a distinct pocket which is bounded below by the cystic duct, above by the floor of the ductus choledochus and laterally by the sides of these structures which are still confluent. This formation is only temporary.

At 20.6 mm. (fig. 50, and fig. 26, *F*) the cystic duct is still more elongated and joins the floor of the ductus choledochus nearly at right angles to it. The gall bladder is shifted downward until its long axis is almost in the vertical plane of the embryo and at right angles to the ductus choledochus. It is drawn out until the sac appears as little more than the expanded end of the cystic duct.

The shifting of the ductus choledochus and the rotation of its anterior end, described on page 371, now takes place, and the cystic duct and a part of the gall bladder share in this displacement. The posterior half of the gall bladder remains almost in the position occupied in the preceding stage or is shifted a little dorsally, while the anterior half and the cystic duct form together an abruptly curved tube which extends to the right and dorsally. In this way there is formed a distinct flexure in the middle part of the gall bladder. This is illustrated by an embryo 28 mm. in length (figs. 51 and 52).

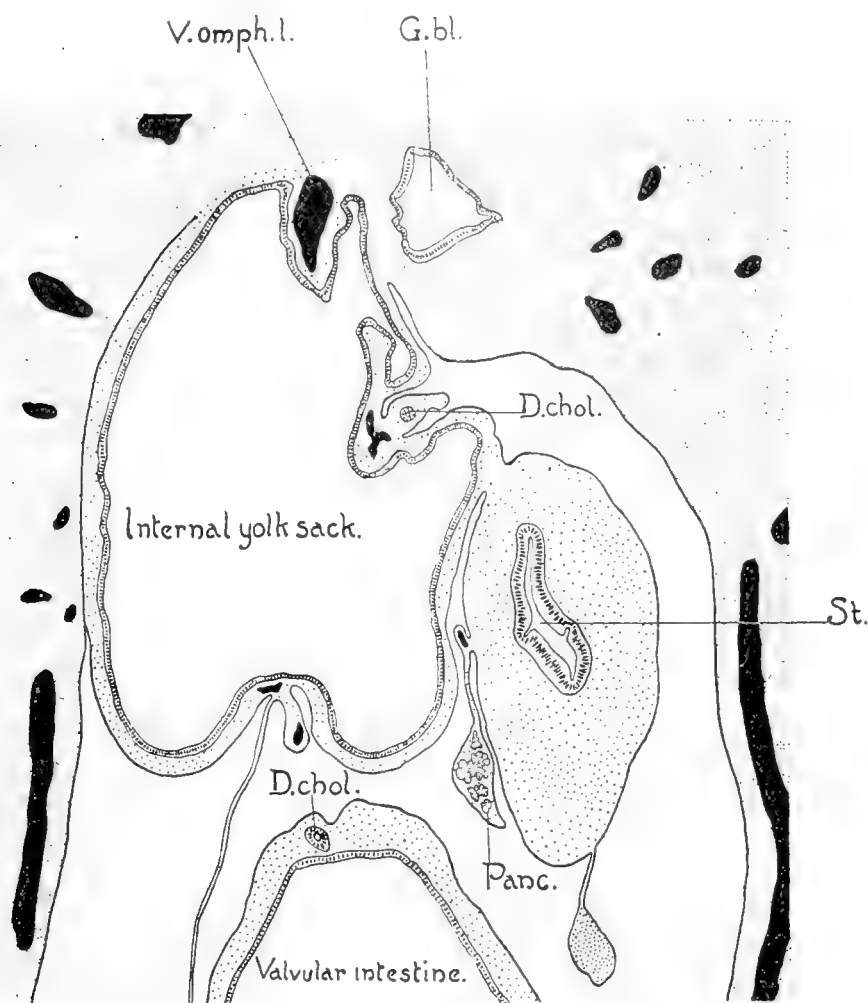


Fig. 27 Frontal section of an *Acanthias* embryo 86 mm. long (H.E.C. 410). $\times 40$. *D.chol.*, ductus choledochus; *G.bl.*, gall bladder; *Panc.*, pancreas; *St.*, stomach; *V.omph.l.*, left omphalo-mesenteric vein.

Along with the changes described above comes a very extensive alteration in both the form and position of the cystic apparatus which is apparently produced by the development of the internal yolk sac. This structure begins to develop from the vitelline duct just internal to the ventral body wall in embryos from 25 to 30 mm. in length. An early stage is seen in section in figure 17. It grows rapidly and its anterior end pushes forward between and below the stomach and left and cystic lobes of the liver on one side and the right lobe of the liver on the other. The frontal section shown in figure 27 illustrates its size and relations in an embryo 60 mm. in length.

In this way the yolk sac presses upon the right side and lower surface of the gall bladder. The first effect of this pressure is to force the posterior part of the gall bladder upward so that this structure retraces in part the path of downward extension which it followed in earlier stages (figs. 22, 23, 24, 25 and 26 *G*). At the same time the gall bladder is elongated and somewhat flattened vertically and its anterior end is pushed upward and forward. At first this only causes an abrupt curve in the cystic duct (fig. 54), but as this process is continued the gall bladder is finally forced anteriorly and dorsally beyond the distal end of the ductus choledochus and the cystic duct proceeds backwards over its dorsal surface to join that structure. In this way the ostium of the cystic duct which was originally in the floor of the ductus choledochus is rotated to its anterior surface and the two ducts together form one continuous tube sometimes called the ductus cystocholedochus, the juncture of the two elements of which is at the ostia of the hepatic ducts. This is also illustrated in figures 22, 23, 24, 25 and 26 *G*.

IV. DEVELOPMENT OF THE DUCTUS CHOLEDOCHUS

The development of the ductus choledochus is better known than that of any other part of the biliary apparatus in elasmobranchs. For our information in regard to the earlier stages we mainly have to thank Hammar ('93), Brachet ('96), Mayr ('97) and Choronschitzky ('00) while the later stages have been most successfully studied in connection with the development of the spiral valve by means of the reconstruction method by Rückert ('96, '97).

The development of this structure in *Acanthias* has been to some extent described incidentally in connection with the account of the early stages of the liver and of the hepatic and cystic ducts and gall bladder in this paper so that only a short summary need be given here.

In *Acanthias*, the ductus choledochus when fully formed is a complex consisting of three embryonic elements: (1) a proximal or posterior segment derived from the floor of the duodenum and

valvular intestine; (2) a middle segment formed from the 'pars ductus lateralis' of the primitive lateral hepatic diverticula; (3) a distal and anterior segment which is differentiated from the posterior portion or 'pars ductus mediana' of the secondary median hepatic pouch. Thus the middle and distal portions of the duct are both derived from the primitive lateral diverticula, at a very early stage and are truly hepatic in origin while the proximal or posterior part is formed from the archenteron at a much later stage after the duodenum and valvular intestine. In the fully formed fish the latter segment forms by far the greater part of the duct being represented by practically all the extra hepatic portion.

The differentiation of the posterior part of the lateral hepatic diverticula into the pars ductus lateralis has already been described in Part I, as well as the early demarkation of the pars ductus mediana from the median hepatic pouch. At 10 mm. the pars ductus lateralis forms the slightly expanded ventral half of the rather constricted segment of archenteron which connects the median hepatic pouch and the gut above it with the yolk-stalk and mid gut posteriorly (figs. 39 and 40). The pars ductus lateralis is thus continuous anteriorly and below with the gall bladder and median pouch and above with the dorsal part of this stalk which later forms the duodenum. On the dorsal surface of the median pouch (fig. 28) is an oblique ridge which extends from its left anterior to right posterior angle and maps off the area of this structure which later becomes the distal part of the ductus choledochus. At 15 mm. (figs. 41 and 42) both the median hepatic pouch and the pars ductus lateralis behind it are completely cut off from the gut above. The latter now forms a short wide duct about 0.1 mm. in length which extends backward a little obliquely from left to right and joins with the floor of the gut a little to the right of the median line. In cross section the duct is equal in diameter to that segment of gut, the duodenum, with which it joins and is triangular in outline with the apex of the triangle directed upward. It thus shows a trace of the pouch-like structure from which it arises but at a little later stage it becomes circular or oval in cross section. The pars duc-

tus mediana is still sac-like and like the duct behind it is inclined a little from left to right.

In 15.5 to 18 mm. embryos the part of the duct derived from the pars lateralis is nearly twice as long as in the above specimen (figs. 45 and 46) and extends directly backward joining the posterior end of the duodenum in the median line. Its diameter is about 0.1 mm., being distinctly less than that of the earlier stage and about one-half that of the gut which it joins. The pars ductus mediana is rapidly taking on a duct-like form although this feature is somewhat obscured by the large ducts which arise from it.

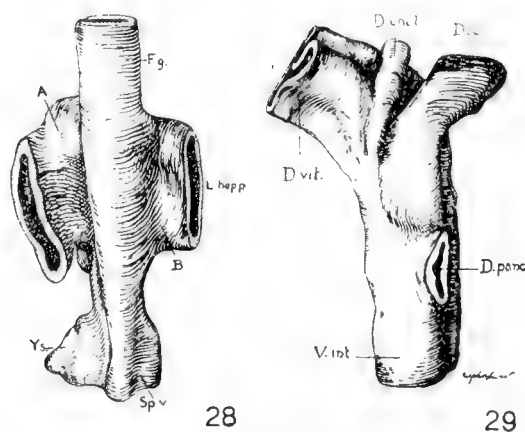


Fig. 28 Dorsal view of a reconstruction of an *Acanthias* embryo 10 mm. long (S.C. 20). $\times 36$. Lateral and anterior views of this model are shown in figures 38 and 39. A, anterior part; B, posterior part of ridge marking the formation of the anterior part of ductus choledochus from the pars ductus medialis of the median liver pouch. F.g., fore gut; L.hepp., lateral hepatic pouches which are cut away dorsally; Sp.v., spiral valve; Y.s., yolk stalk.

Fig. 29 Reconstruction of the mid gut region of an *Acanthias* embryo 20.5 mm. long (S.C. 5). $\times 50$. Duo., duodenum; D.chol., ductus choledochus; D.panc., pancreatic duct; D.vit., vitelline duct; V.int., valvular intestine.

In the stages which now follow, as is shown in figures 51, 52 and 54, the duct rapidly increases in length due to the elongation of its extra-hepatic portion. This growth seems to be derived from the floor of the digestive tube for there extends in the floor of the gut posterior to the ostium of the duct a deep groove which is lined with epithelium of the same nature as that of the duct itself. At 15.5 mm., the duct joins the gut near the posterior end of the duodenum. At 20.5 mm., as is shown in figure 29, this opening lies at the point where the duodenum becomes continuous

with the valvular intestine in the angle between the latter structure and the vitelline duct. The duct lies below and to the right of the median line of the duodenum, but as this structure is continuous only with the left side of the valvular intestine (the right side of which is continuous with the vitelline duct) the ductus choledochus joins the valvular intestine directly in the median line of the gut.

From this time to until the embryo reaches a length of approximately 25 mm. the duct grows steadily backward and its ostium remains in the median line. It grows past the opening of the vitelline duct passing it to the left but never overtakes the ostium of the pancreatic duct.

When the embryo reaches the length of about 25 mm., however, the duct becomes involved in the twisting process of the spiral valve and is carried to the left. At 28 mm. its opening is in the middle of the right side of the valvular intestine and at 37 to 48 mm. it lies at the junction of the superior and right surfaces. In embryos 60 to 80 mm. long, the duct enters the intestine on its dorsal surface at the edge of the first turn of the spiral valve, and in the new-born fish the opening lies at the junction of the left side with the dorsal surface of the intestine. These changes in position as well as the comparative diameter of the duct at different stages are shown in table 2.

Along with the elongation of the ductus choledochus come several modifications of its position besides the posterior shifting just described. The intrahepatic portion is affected by the growth of the internal yolk sac and by the vascular changes already discussed in connection with their effect upon the position of the hepatic and cystic ducts. This part of ductus choledochus is first arched upward in a stiff almost semi-circular curve by the formation of a venous sinus below it and its extreme anterior end is rotated to the right. These changes are shown in figures 51, 52 and 54. In later stages and in the new-born fish these changes are less noticeable, being probably compensated by the growth of the hepatic parenchyma, but in the new-born fish as well as the adult the distal end of the duct lies distinctly to right of the median line. As the duodenal flexure is developed, the

middle part of the duct is also directed sharply ventrally along with the change in position of the segment of the gut which it joins. This ventral curve appears in embryos of 18 to 20 mm. and is at first a gradual one but becomes sharper and more pro-

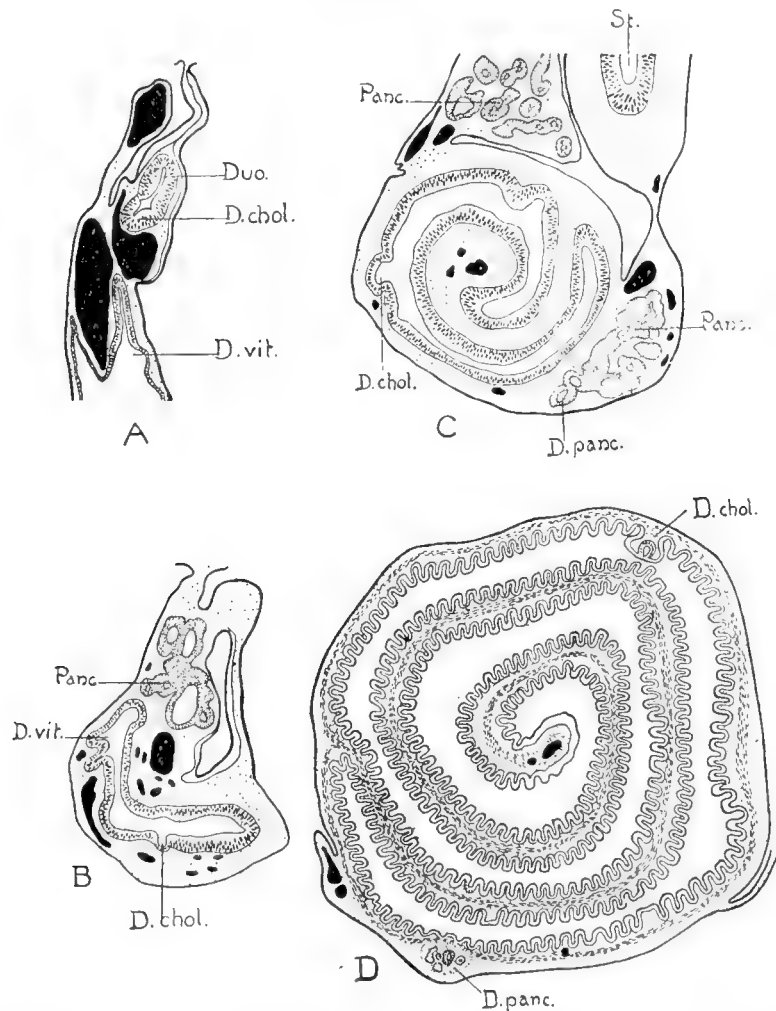


Fig. 30 A series of transverse sections of *Acanthias* embryos of different ages at the level of the ostium of the ductus choledochus. *A*, embryo 15.5 mm. long (S.C. 1); *B*, embryo 20.6 mm. long (H.E.C. 1494); *C*, embryo 32.2 mm. long (H.E.C. 1662); *D*, embryo 180 mm. long (S.C. 51) all except *D* \times 28; *D* \times 8. *D.chol.*, ductus choledochus; *D.panc.*, pancreatic duct; *D.vit.*, vitelline duct; *Duo.*, duodenum; *Panc*, pancreas; *St.*, stomach.

nounced in older embryos. In the course of this curve the duct is also pushed to the right by the vitelline vein which lies beside it. Minor short flexures appear in later stages near the anterior and posterior ends of the duct and in its duodenal curve as well. They are not constant in position, shape or number. The duct

is much reduced in diameter between the time when it is first formed and when it begins its growth backward along the base of the valvular intestine. After this phase begins there is but little change in caliber. In the intrahepatic and preduodenal regions the duct is circular in trans-section, posteriorly it is flattened transversely until elongately oval in trans-section.

Table 2 shows numerically the growth of the duct and the changing relations of its ostium. Some error is doubtless introduced in the calculation of the length of the duct from cross sections as is done here, but *Acanthias* embryos of 18 mm. and over shrink comparatively little in imbedding and that shrinkage seems to be symmetrical so that the value of the figures is not seriously impaired. The 'extra-hepatic length' is taken from the point where the duct turns sharply downward immediately after leaving the liver to the point where its lumen becomes continuous with that of the gut. It is not possible to determine this point exactly in specimens under 20 mm. in length so only the total length of the duct has been given in such cases.

TABLE 2

Growth and relations of the ductus choledochus

DESIGNATION	LENGTH OF EM- BRYOS	LENGTH OF DUCT			DIAM- ETER OF DUCT	RELATION OF OSTIUM TO OSTIUM OF VITEL- LINE DUCT	RELATION OF OSTIUM TO OSTIUM OF PAN- CREATIC DUCT	POSITION OF OSTIUM IN INTESTINAL WALL
		Intra hepatic	Extra hepatic	Total				
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	
H.E.C. 227...	15.0			0.11	0.15	0.23 ant.	0.40 ant.	Entire ventral surface
S.C. 1.....	15.5			0.22	0.11	0.20 ant.	0.32 ant.	Entire ventral surface
S.C. 2.....	19.0			0.16	0.9	0.14 ant.	0.33 ant.	Entire ventral surface
H.E.C. 1494..	20.6	0.22	0.18	0.40	0.8	Same plane.	0.12 ant.	Mid-line of ventral sur- face
H.E.C. 1492..	24.7	0.10	0.32	0.42	0.8	0.10 post.	0.35 ant.	Junction right and mid- dle thirds of ventral surface
H.E.C. 1357..	28.0	0.70	0.62	0.72	0.5	0.40 post.	0.25 ant.	Junction lower and mid- dle thirds of right sur- face
H.E.C. 1652..	32.2	0.16	0.86	1.02	0.5	0.60 post.	0.29 ant.	Junction lower and mid- dle thirds of right sur- face
H.E.C. 363...	37.0	0.36	1.03	1.44	0.7	0.80 post.	0.46 ant.	Junction right and dorsal surfaces.
S.C. 11.....	47.3	0.72	1.10	1.82	0.7	0.91 post.	1.76 ant.	Junction right and dor- sal surfaces
H.E.C. 1882..	95.0	1.20	5.0	6.2	0.7	2.4 post.	1.2 ant.	Mid-line of dorsal surface

V. GENERAL SUMMARY

The observations here recorded may be summarized as follows:

1. The liver arises in *Acanthias* embryos of from 20 to 25 segments as a pair of shallow lateral diverticula from the lateral walls of the ventral half of the gut. These diverticula extend both behind and in front of the anterior wall of the yolk-stalk.

2. The growth of the fore gut posteriorly through the coalescence of the lateral walls of the yolk-stalk causes the lateral hepatic diverticula to lie mainly in front of the anterior wall of the yolk-stalk in later stages.

3. The median ventral liver pouch described by Balfour and others is, in *Acanthias* at least, a secondary structure produced by the fusion of the anterior ends of the primary lateral diverticula.

4. Three distinct secondary parts are derived from each primitive lateral hepatic diverticulum. The anterior portion goes to the median hepatic pouch. The upper part of the middle portion forms the lateral hepatic pouch. The posterior part goes to form a posterior connecting segment between the liver and gut. This has been termed the *pars ductus lateralis* and later becomes a part of the *ductus choledochus*.

5. At an early stage the liver shares in the left to right rotation which produces the spiral valve in the intestine and the lateral vitelline groove in the yolk stalk.

6. The median hepatic pouch is somewhat differentiated into two parts: an anterior one, the *pars hepatica mediana* to which the lateral pouches are mainly attached and which gives rise to hepatic trabeculae, and a posterior one, the *pars ductus mediana*, which forms the anterior part of the *ductus choledochus*.

7. The anterior part of the left hepatic duct is formed from the left hepatic pouch and the left part of the *pars hepatica mediana*. The anterior part of the right hepatic duct is formed from the right hepatic pouch alone.

8. The hepatic ducts and *ductus choledochus* are very markedly rotated to the right about a vertical axis in a comparatively late stage. This rotation is probably due to the great growth of the left omphalo-mesenteric vein and the formation of a venous

sinus below and to the left of the anterior end of the ductus choledochus.

9. The difference in size of the right and left omphalo-mesenteric veins may be due to the longitudinal rotation of the gut mentioned in section 5 of this summary. By this rotation the space between the left hepatic pouch and the median pouch and gut is somewhat increased while the corresponding space on the opposite side is decreased.

10. A third shifting of the duct system and the gall bladder forward and upward occurs at a much later stage. It is probably brought about through the great increase in size of the internal yolk sac.

11. The minor hepatic ducts arise as the elongated pedicles of definitely placed groups of hepatic tubules. These tubule groups are differentiated at the time when the liver loses its dorsal connection with the fore gut.

12. The variations in position of the minor hepatic ducts in the adult depend upon the degree of shifting of these tubule groups at the time when the main hepatic pouches are differentiated into hepatic ducts and when the rotations mentioned in sections 5 and 8 take place.

13. The gall bladder appears much later than do the primary lateral hepatic diverticula. It arises posterior to the hepatic anlage as a distinct evagination of the gut at the juncture of the floor of the fore gut and anterior wall of the yolk sac, and its intimate connection with the liver duct system is acquired secondarily.

14. The gall bladder loses its dorsal and posterior connection with the gut and is shifted forward and downward until its greatest axis is in the transverse plane of the body. In this way its connection with the ductus choledochus comes to lie in front of that of the lateral hepatic ducts.

15. Subsequently the gall bladder is again shifted upward and forward so that the cystic duct passes backward and downward to join the ductus choledochus. This is brought about by the agency mentioned in section 10.

16. The ductus choledochus is formed from three distinct elements: the anterior part from the pars ductus mediana, a deriva-

tive of the secondary median hepatic pouch; the middle part from the pars ductus lateralis, a derivative of the posterior part of the primary hepatic diverticula, and a posterior part formed from the floor of the duodenum and valvular intestine.

17. Finally, the results of this study may be summarized in tabular form as given below, starting with the sources of the hepatic apparatus on the left hand side, and ending with end results of their evolution on the right. In this table the minor ducts are arranged in the way that they seem to occur most frequently in the late embryo or new-born fish. To this table should be added the statement that all those embryonic structures which are included under the term 'pars hepatica' give rise to hepatic trabeculae as well as to the conducting structures which are listed here.

TABLE 3
Summary of the origin of hepatic structures in Acanthias

[illegible]

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PLATES

PLATE 1

EXPLANATION OF FIGURES

- 31 Lateral view of a reconstruction of a part of the archenteron of an *Acanthias* embryo 6.4 mm. long (S.C. 19). $\times 100$.
32 Anterior view of the reconstruction seen in figure 31. $\times 100$.
33 Lateral view of a reconstruction of the hepatic region of an *Acanthias* embryo 7.5 mm. long (H.E.C. 1503). $\times 75$.
34 Anterior view of the reconstruction seen in figure 33.
35 Ventral view of a reconstruction of the hepatic region of an *Acanthias* embryo 9 mm. long (H.E.C. 1495). $\times 75$.
36 Anterior view of the reconstruction seen in figure 35. $\times 75$.
37 Ventral view of a reconstruction of the hepatic region of an *Acanthias* embryo 7.5 mm. long (S.C. 15). $\times 75$.
38 Anterior view of the reconstruction seen in figure 37. $\times 75$.

B.en., blastodermic entoderm
D.cyst., cystic duct
F.g., fore gut
G.bl., gall bladder
Hep.d., hepatic diverticulum

L.hep.p., lateral hepatic pouch
M.hep.p., median hepatic pouch
T.a., anlagen of hepatic tubules
Y.s., anterior wall of yolk stalk

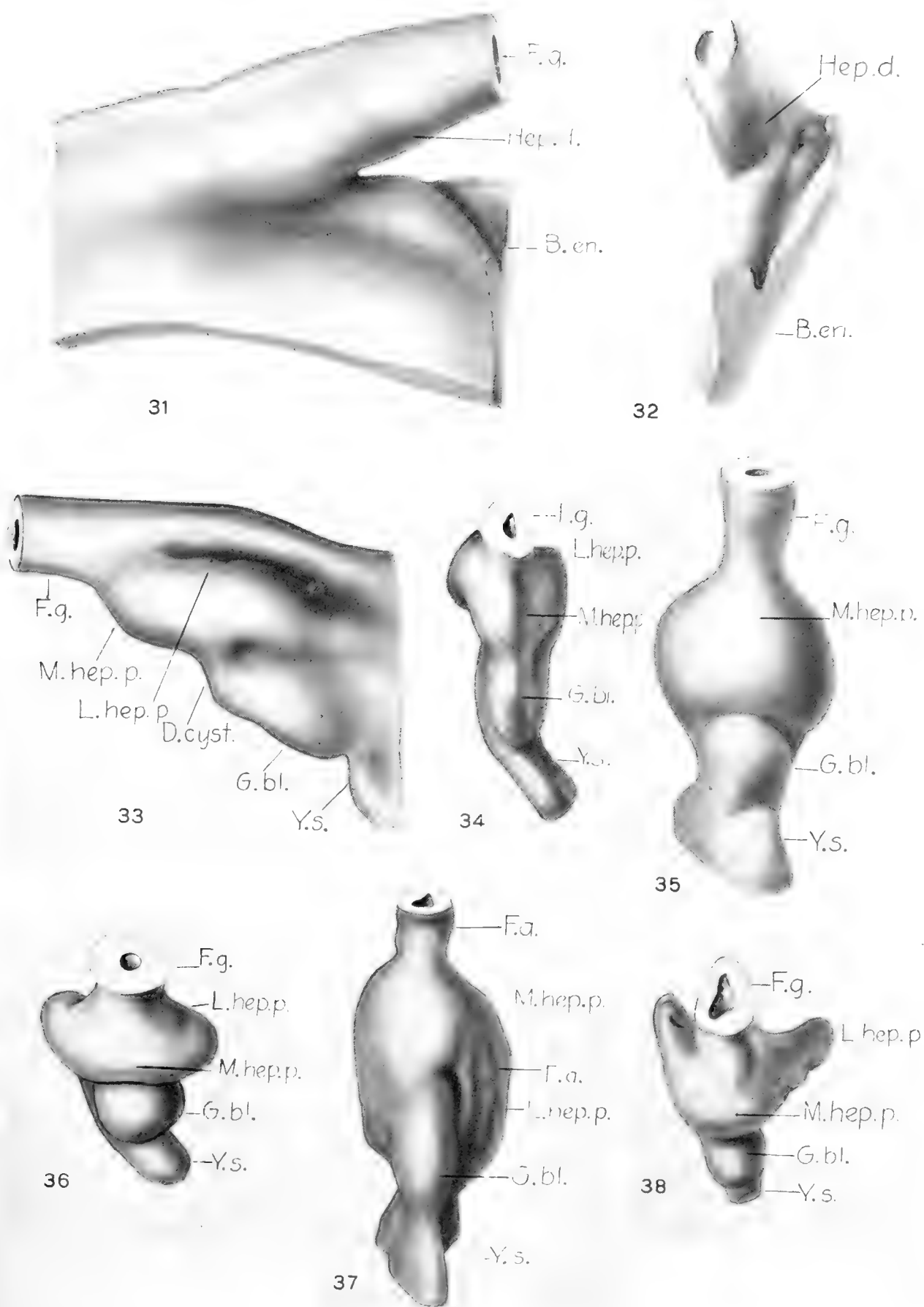


PLATE 2

EXPLANATION OF FIGURES

39 Anterior view of a reconstruction of the liver of an *Acanthias* embryo
10 mm. long (S.C. 20). $\times 100$.

40 Left lateral view of the same reconstruction. $\times 100$.

F.g., fore gut

G.bl., gall bladder

L.hep.p., lateral hepatic pouch

M.hep.p., median hepatic pouch

P.hep.m., pars hepatica medialis

T.a., anlagen of hepatic tubules

Y.s., yolk stalk

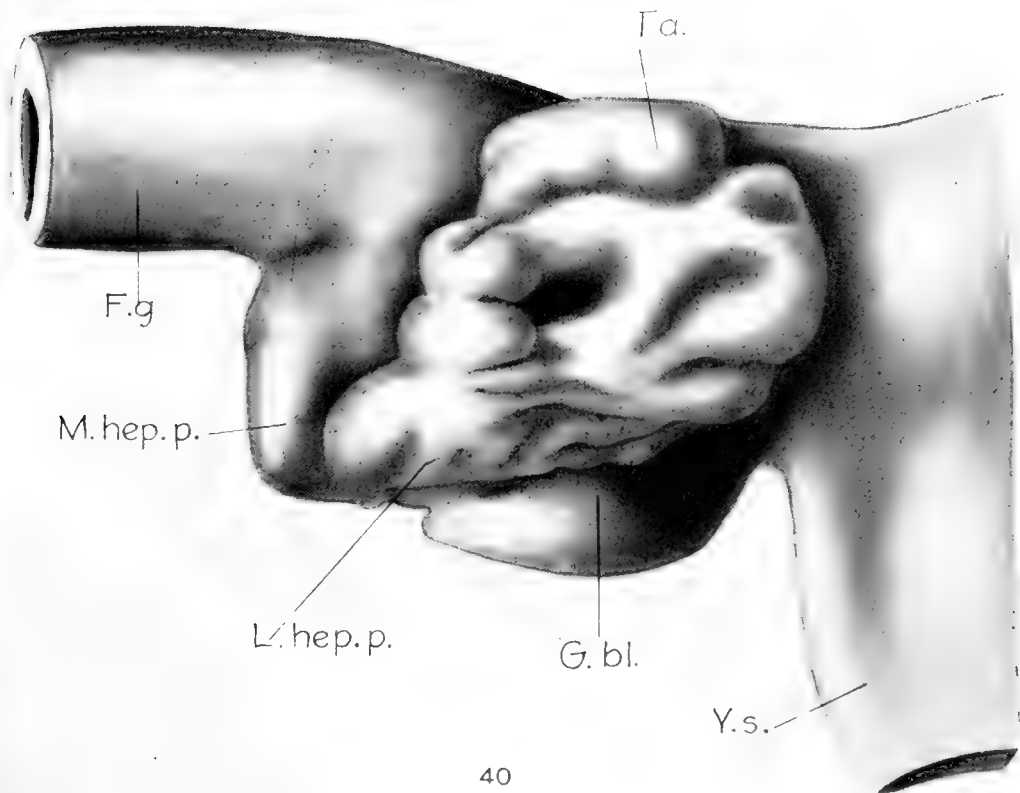
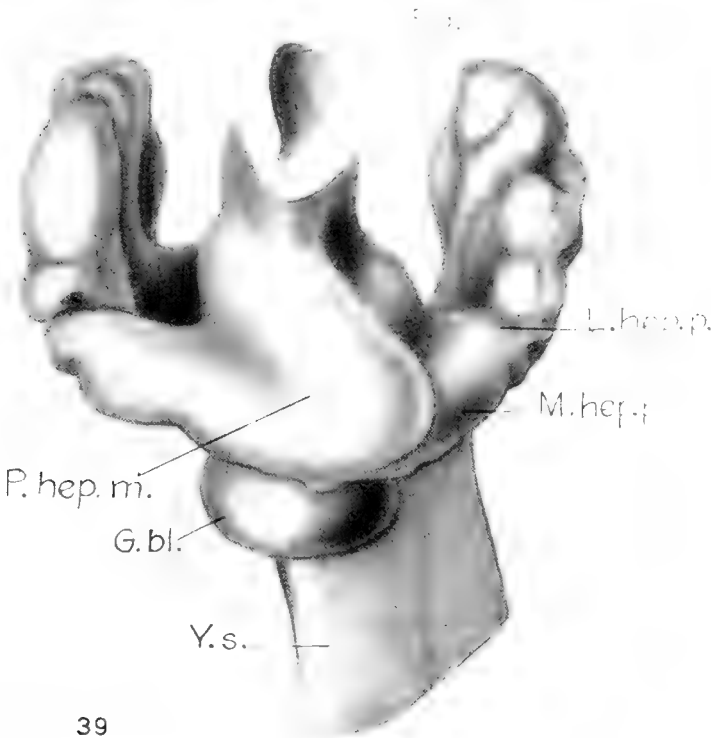


PLATE 3

EXPLANATION OF FIGURES

41 Right lateral view of a reconstruction of the liver of an *Acanthias* embryo 15 mm. long (H.E.C. 227). $\times 150$.

42 Left lateral view of the same reconstruction.

D.chol., ductus choledochus

G.bl., gall bladder

L.hep.p., left hepatic pouch

R.hep.p., right hepatic pouch

St., stomach

T.a.l., anterior left tubule group

T.a.r., anterior right tubule group

T.l.m., left medial tubule group

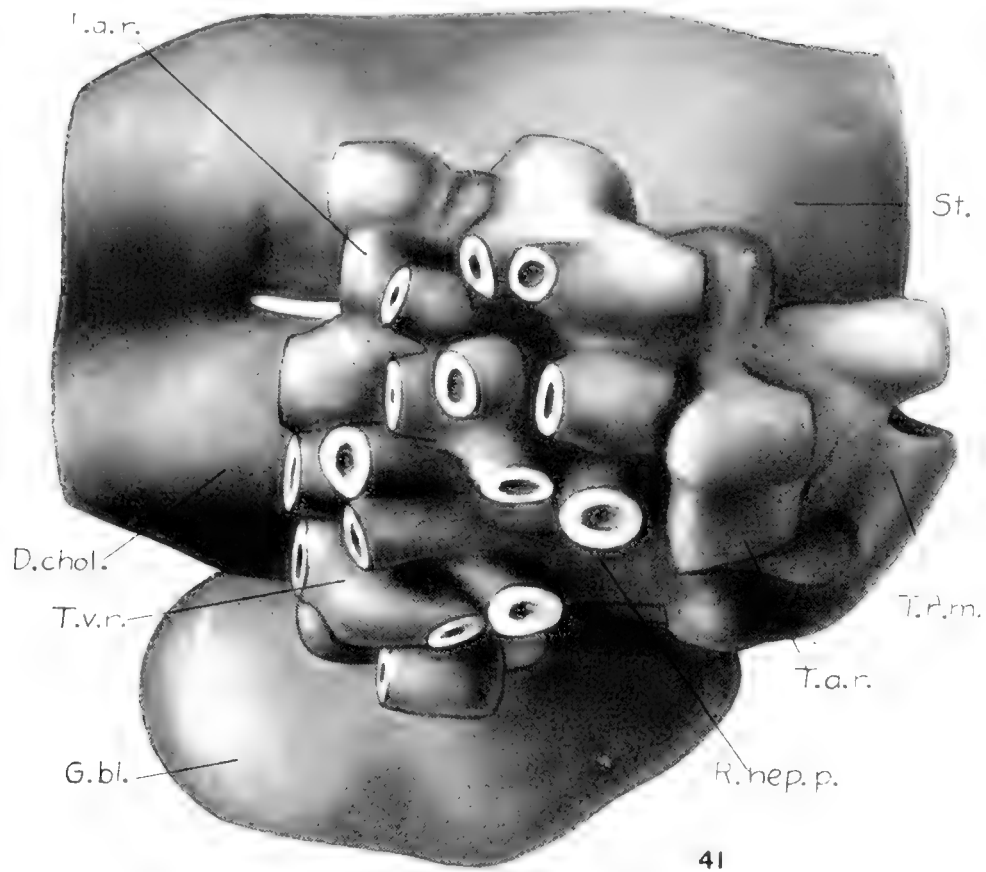
T.d.l., dorsal cluster, posterior left tubule group

T.v.l., ventral cluster, posterior left tubule group

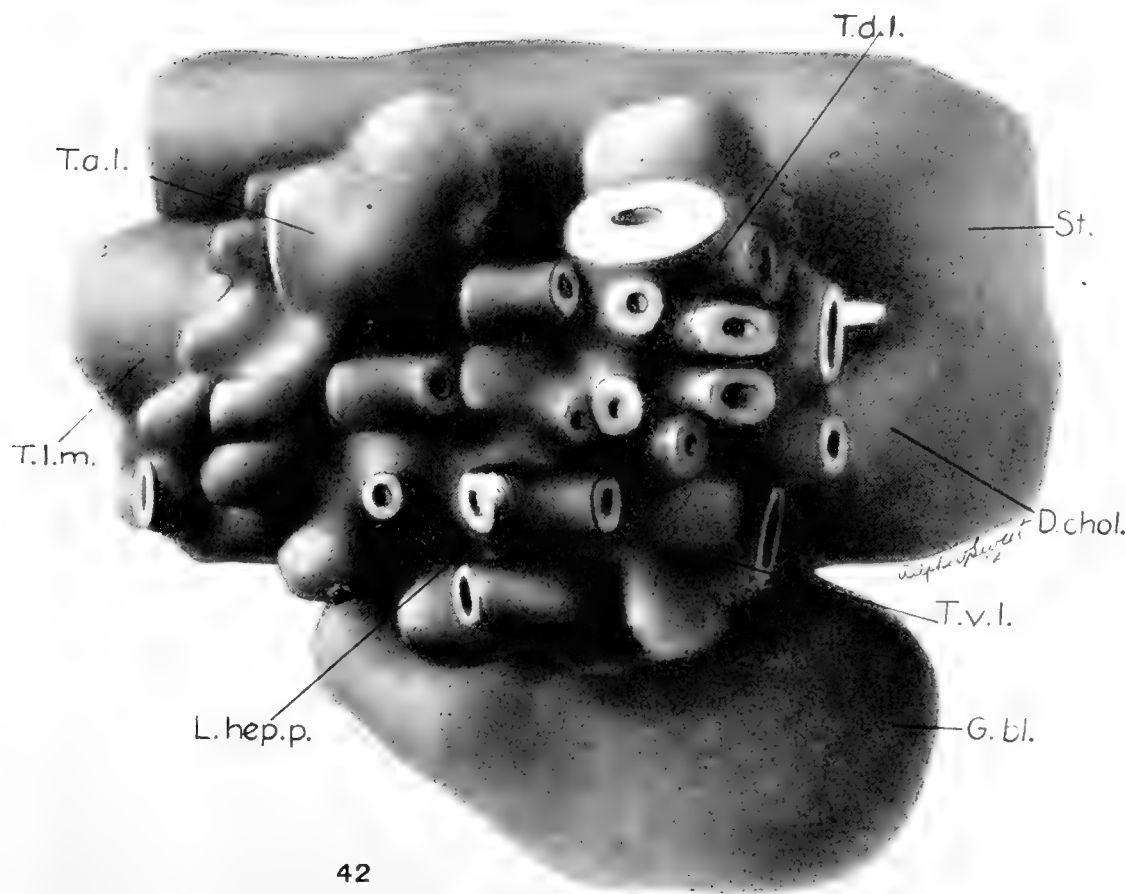
T.r.m., right medial tubule group

T.d.r., dorsal cluster, posterior right tubule group

T.v.r., ventral cluster, posterior right tubule group



41



42

PLATE 4

EXPLANATION OF FIGURES

43 Anterior view of a reconstruction of the liver of an *Acanthias* embryo 15.5 mm. long (S.C. 1). $\times 120$.

44 Anterior view of the reconstruction illustrated in figures 41–42. $\times 150$.

G.bl., gall bladder

St., stomach

T.a.l., *T.l.a.*, anterior left tubule group

T.a.r., anterior right tubule group

T.d.l., dorsal cluster, posterior left tubule group

T.d.r., dorsal cluster, posterior right tubule group

T.l.m., left medial tubule group

T.p.l., posterior left tubule group—the separate clusters cannot be seen in this view of figure 43.

T.r.m., right medial tubule group

T.v.l., ventral cluster, posterior left tubule group

T.v.r., ventral cluster, posterior right tubule group

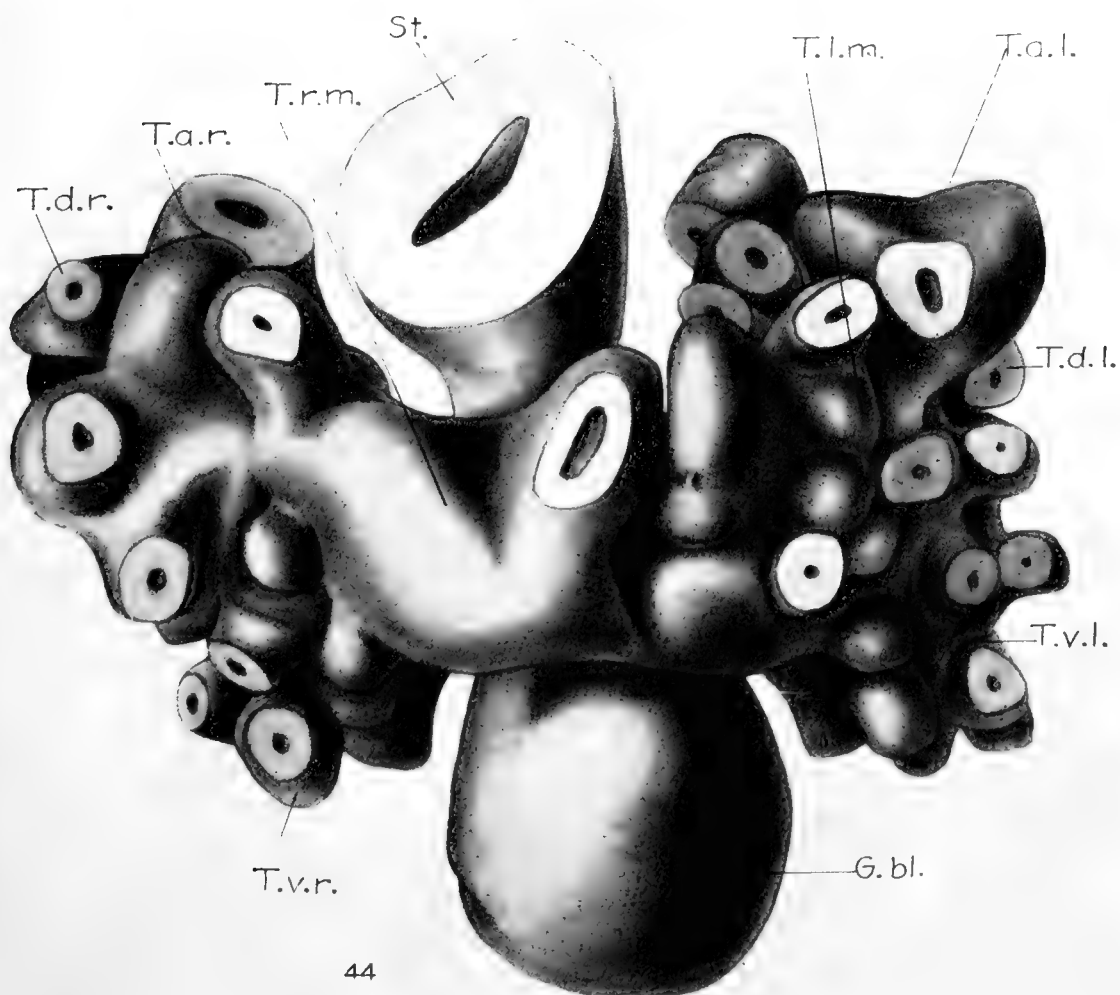
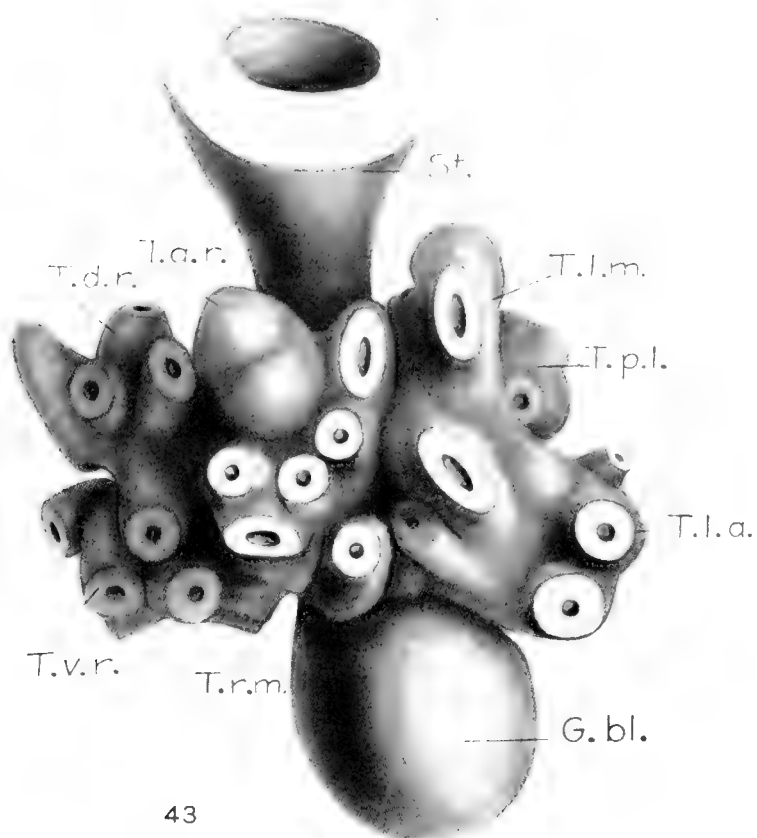


PLATE 5

EXPLANATION OF FIGURES

45 Left lateral view of a reconstruction of the liver of an *Acanthias* embryo 15.5 mm. long (S.C. 1). $\times 100$.

46 Right lateral view of a reconstruction of the same reconstruction. $\times 100$.

D.chol., ductus choledochus

D.cyst., cystic duct

Duo., duodenum

G.bl., gall bladder

St., stomach

T.a.l., anterior left tubule group

T.a.r., anterior right tubule group

T.d.l., dorsal cluster, posterior left
tubule group

T.d.r., dorsal cluster, posterior right
tubule group

T.l.m., left medial tubule group

T.r.m., right medial tubule group

T.v.l., ventral cluster, posterior left
tubule group

T.v.r., ventral cluster, posterior right
tubule group

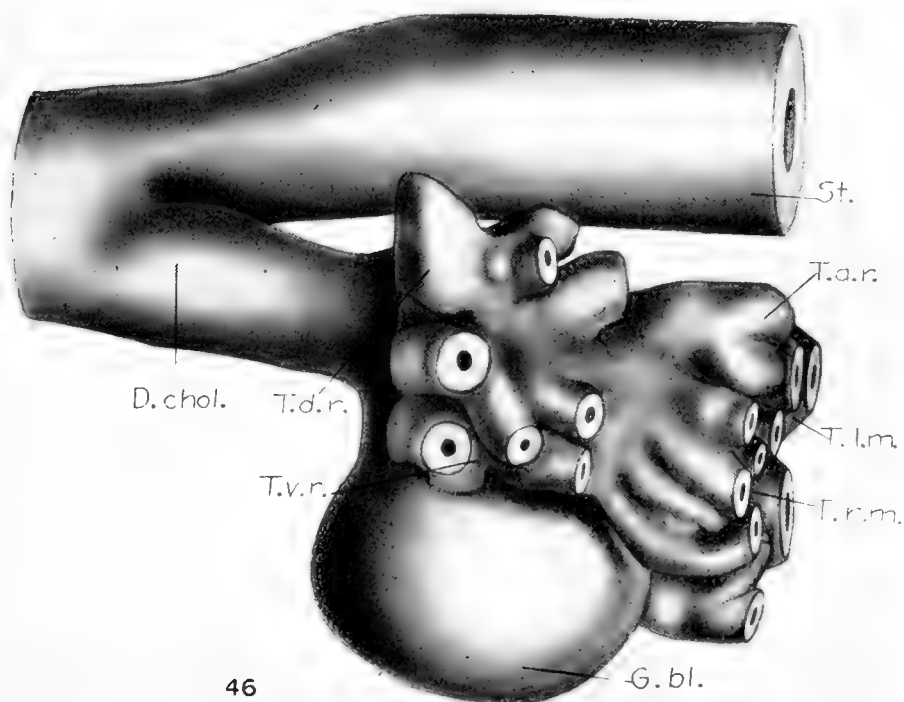
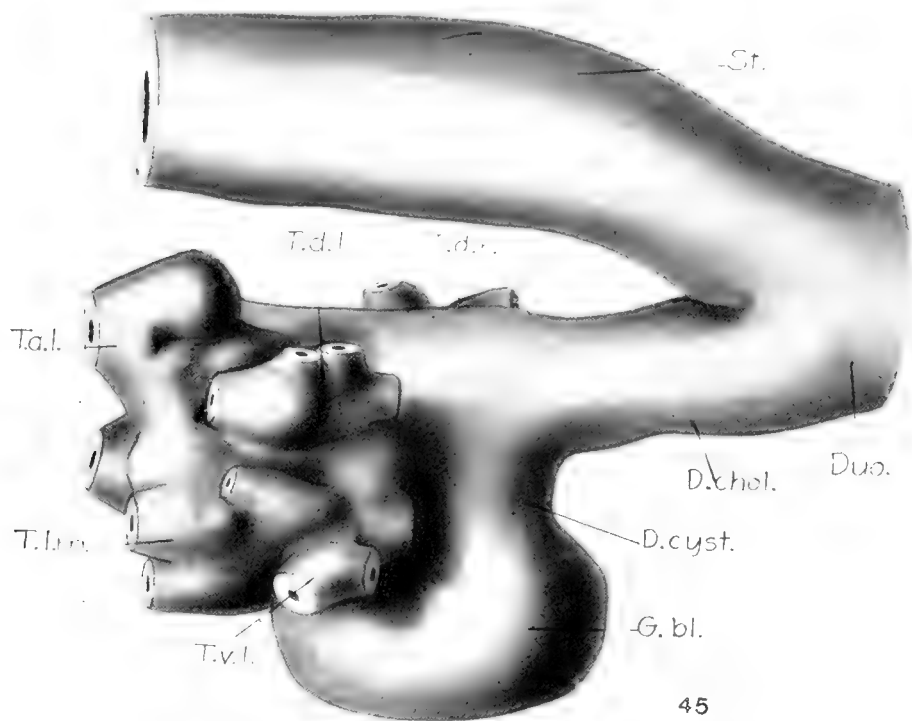


PLATE 6

EXPLANATION OF FIGURES

47 Left lateral view of a reconstruction of the liver of an *Acanthias* embryo 20.5 mm. long (S.C. 5). $\times 75$.

48 Right lateral view of the same object. $\times 75$.

D.chol., ductus choledochus

D.cyst., cystic duct

G.bl., gall bladder

L.hep.d., left hepatic duct

T.a.l., anterior left tubule group

T.a.r., anterior right tubule group

T.d.l., dorsal cluster, posterior left
tubule group

T.d.r., dorsal cluster, posterior right
tubule group

T.v.l., ventral cluster, posterior left
tubule group

T.v.r., ventral cluster, posterior right
tubule group

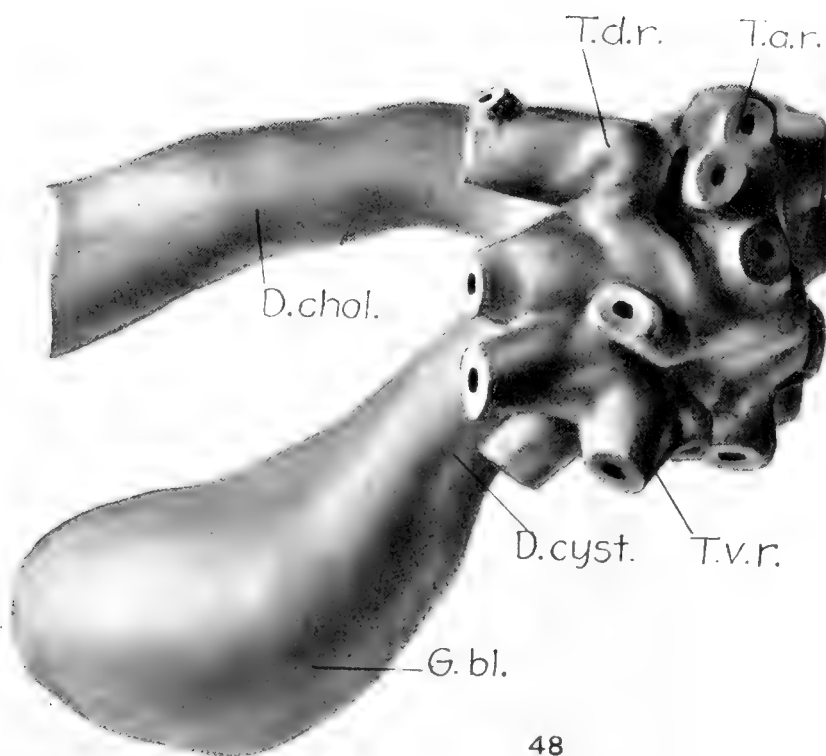
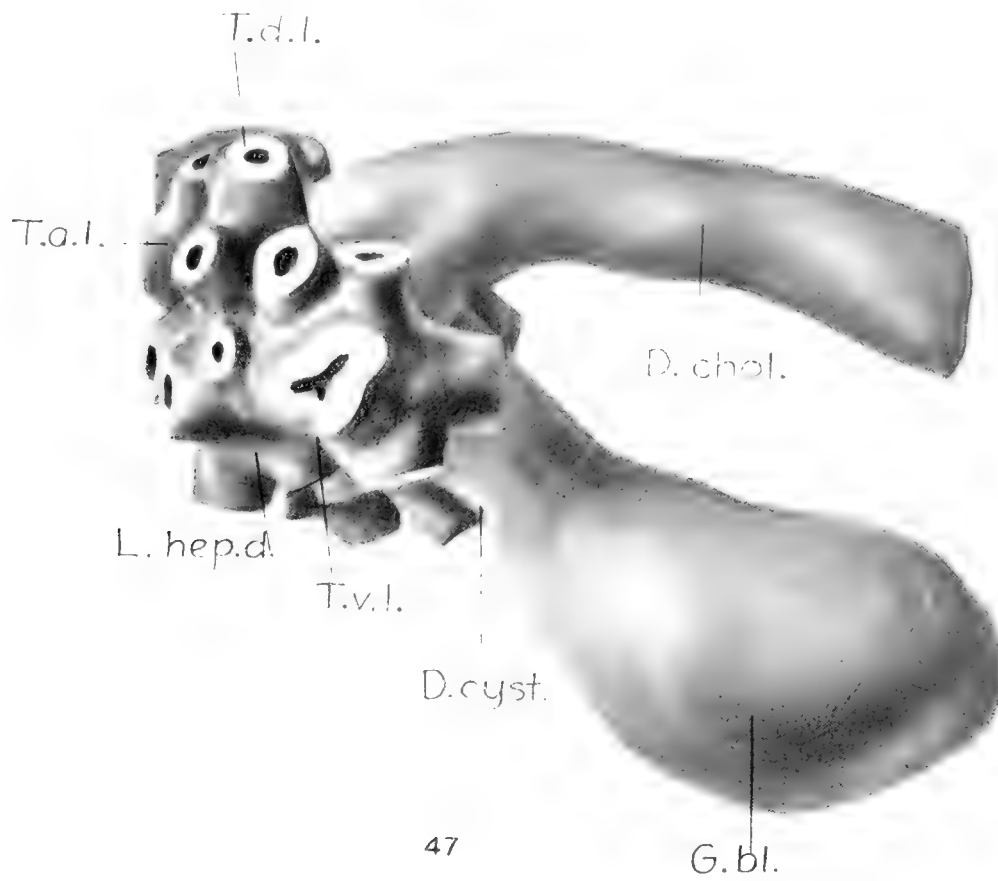


PLATE 7

EXPLANATION OF FIGURES

49 Anterior view of the reconstruction shown in figures 47 and 48. $\times 75$.

50 Anterior view of a reconstruction of the gall bladder and liver ducts of an *Acanthias* embryo 20.6 mm. long (H.E.C. 1494). $\times 75$.

D.chol., ductus choledochus

D.cyst., cystic duct

D.hep.l., left hepatic duct

D.hep.r., right hepatic duct

G.bl., gall bladder

T.a.l., anterior left hepatic ramus or tubule group

T.a.r., anterior right ramus or tubule group

T.p.l., dorsal cluster, posterior left tubule group, which later forms the posterior left ramus

T.d.r., dorsal cluster, posterior right tubule group

T.l.m., left medial ramus or tubule group

T.r.m., right medial ramus or tubule group

T.v.l., minor tubules of ventral left tubule group

T.v.r., minor tubules of ventral right tubule group

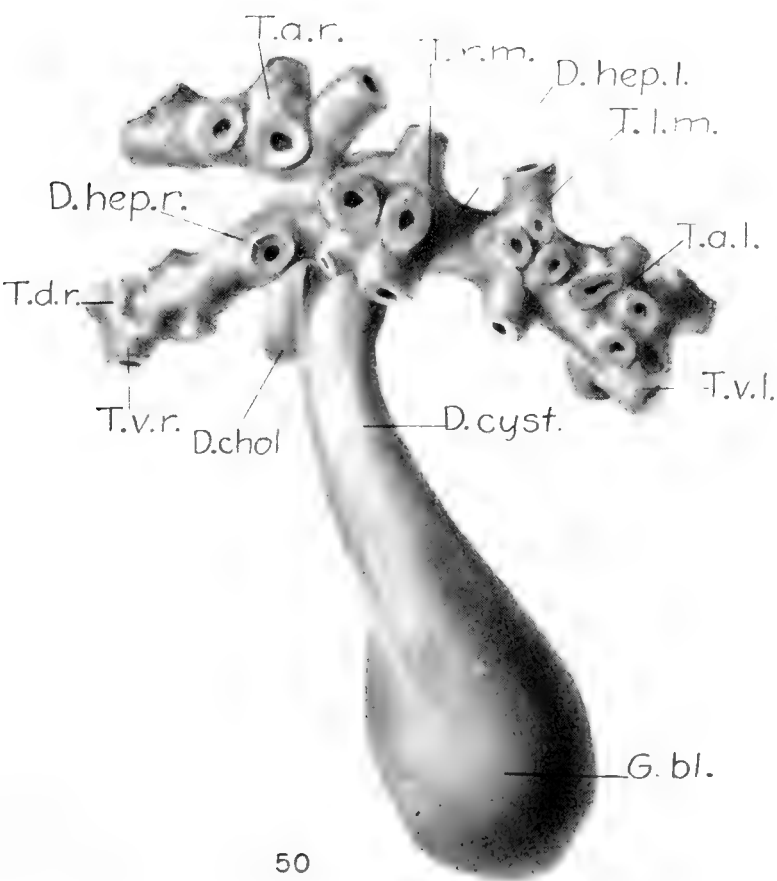
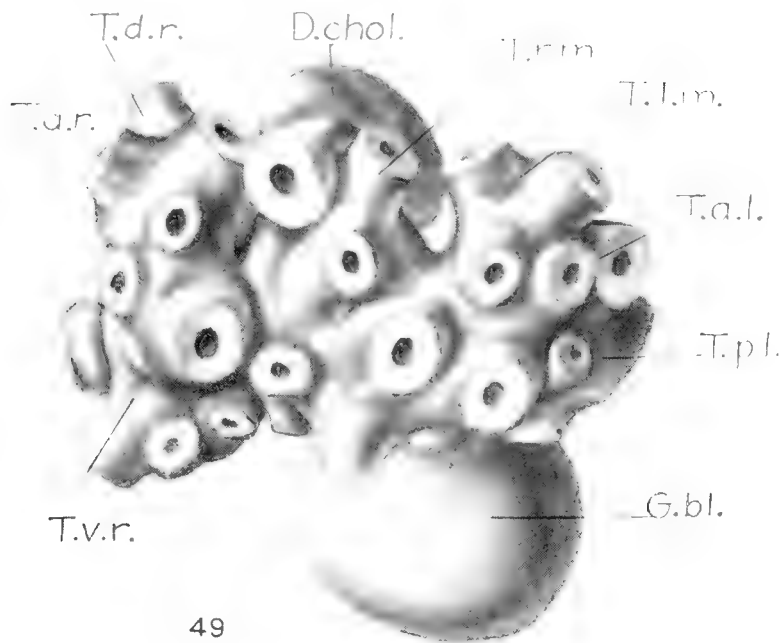


PLATE 8

EXPLANATION OF FIGURES

51 Left lateral view of a reconstruction of a part of the gut and the liver ducts and gall bladder of an *Acanthias* embryo 28 mm. long (S.C. 6). $\times 50$.

52 Antero-ventral view of the same reconstruction. $\times 50$.

D.chol., ductus choledochus

D.cyst., cystic duct.

D.hep.l., left hepatic duct

D.hep.r., right hepatic duct

D.panc., pancreatic duct

D.vit., vitelline duct

Duo., duodenum

G.bl., gall bladder

Panc., pancreas

R.a.l., anterior left hepatic ramus

R.a.r., anterior right ramus

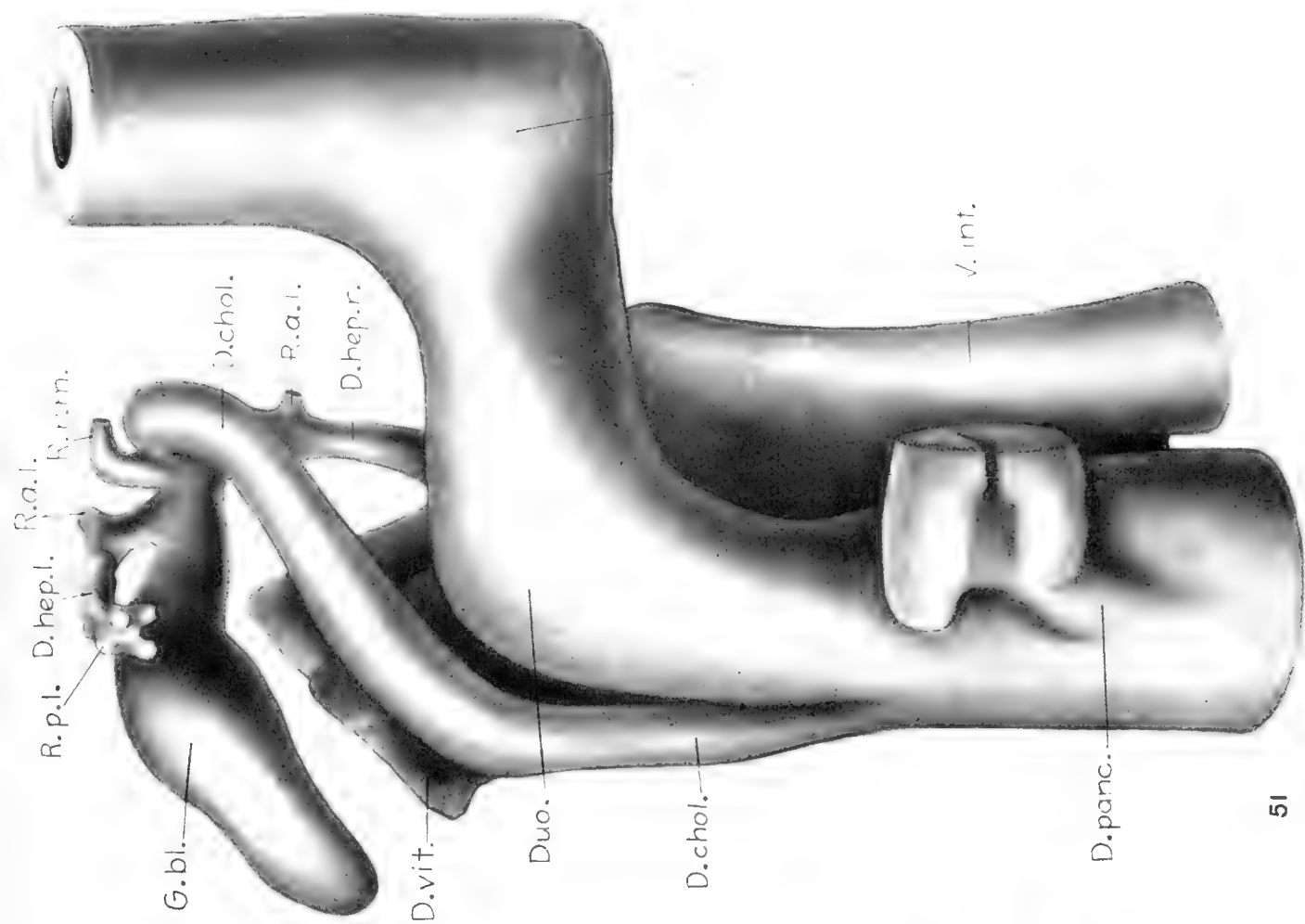
R.l.m., left medial ramus

R.p.l., posterior left ramus

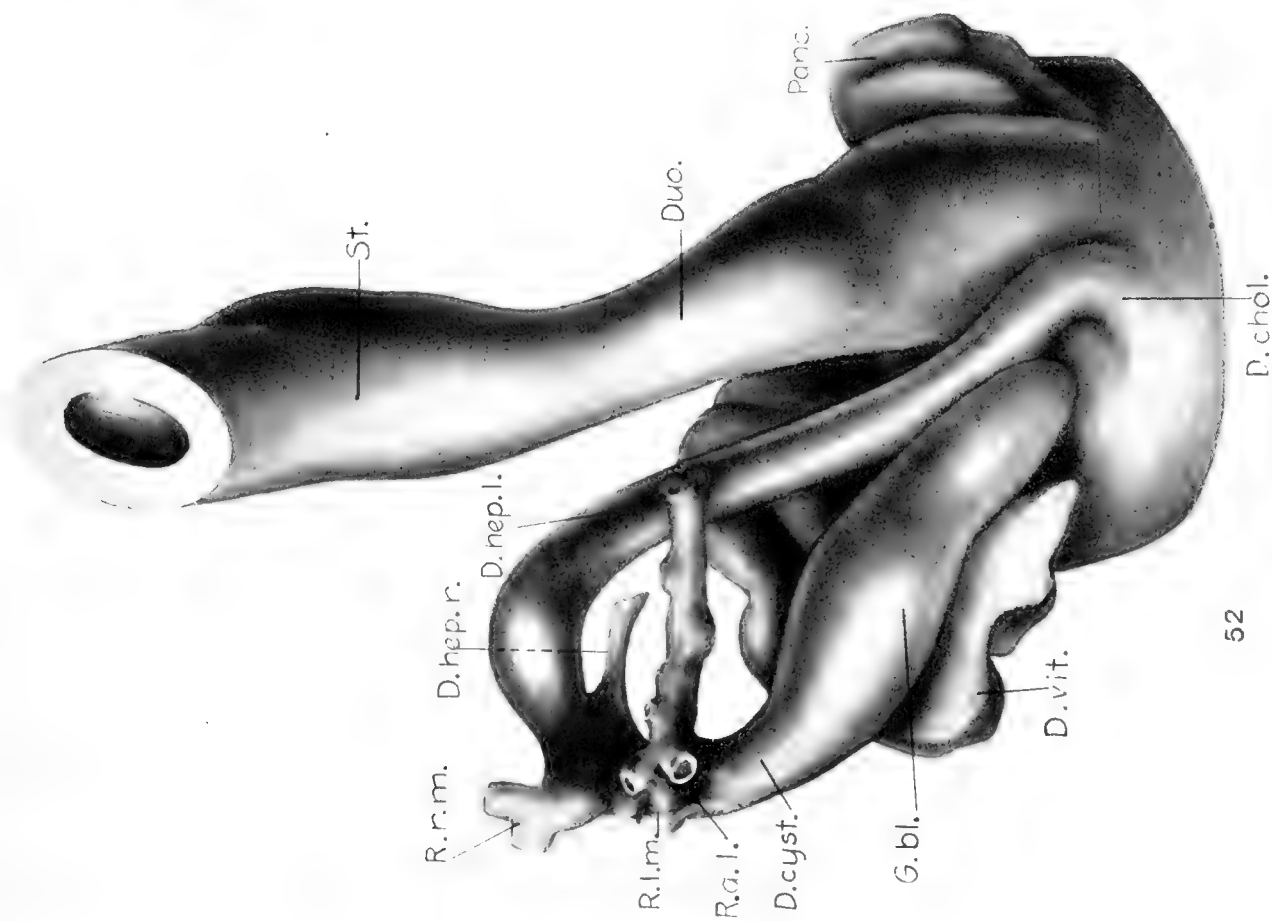
R.r.m., right medial ramus

St., stomach

V.int., valvular intestine



51



52

PLATE 9

EXPLANATION OF FIGURES

53 Anterior view of a reconstruction of the gall bladder and liver ducts of an *Acanthias* embryo 33.1 mm. long (S.C. 8). $\times 50$.

54 Ventral view of a reconstruction of the gall bladder, liver ducts, and a part of the gut of the same embryo. $\times 50$.

D.chol., ductus choledochus

D.cyst., cystic duct

D.hep.l., left hepatic duct

D.hep.r., right hepatic duct

D.vit., vitelline duct

Duo., duodenum

G.bl., gall bladder

R.a.l., *R.a.m.*, anterior left hepatic ramus

R.a.r., anterior right ramus

R.d.l., dorsal posterior left ramus

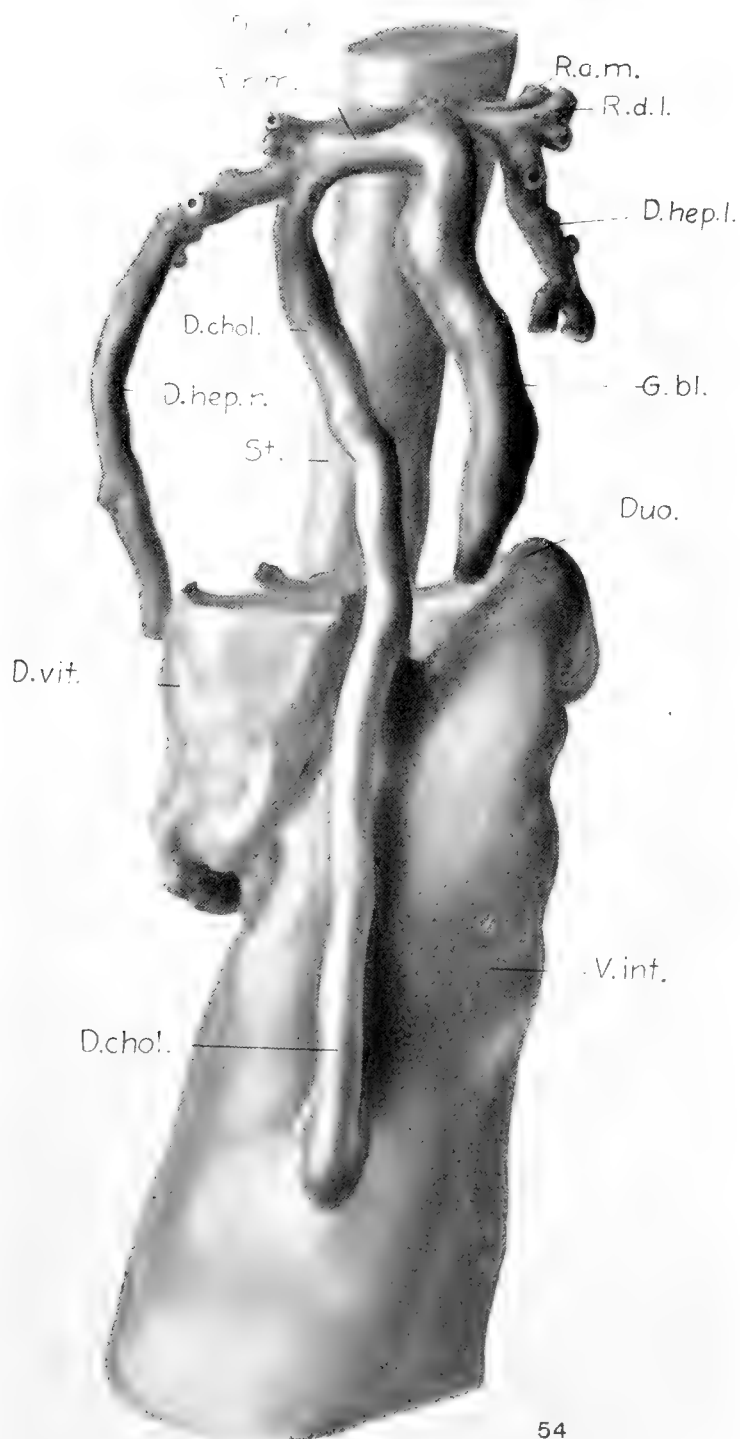
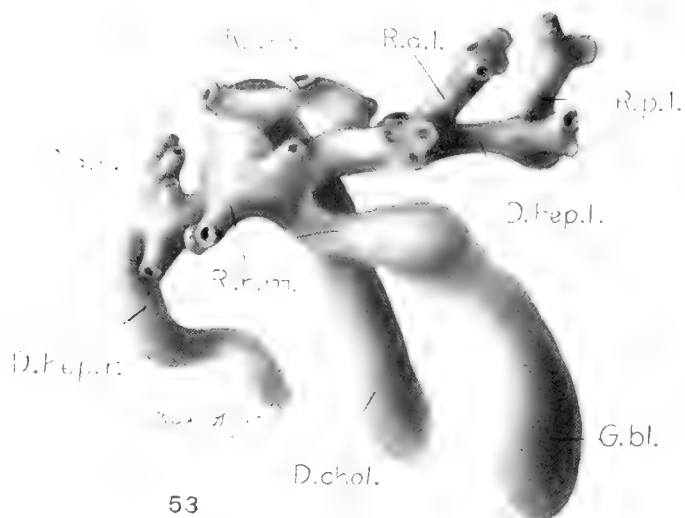
R.l.m., left medial ramus

R.p.l., posterior left ramus

R.r.m., right medial ramus

St., stomach

V.int., valvular intestine





THE FASCICULUS CEREBRO-SPINALIS IN THE ALBINO RAT

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TEN FIGURES

It is well known that the fasciculus cerebro-spinalis, more commonly called the cortico-spinal or pyramidal tract, does not occupy the same position in the spinal cord in all orders of mammals. But, according to the animal which is being studied, it may be found in any one or two of the three funiculi of the cord. From its constant position in the ventro-medial portion of the medulla it passes in the rat to the opposite posterior funiculus of the spinal cord; while in the mole it runs without decussation into the anterior funiculus of the same side. In the cat it decussates into the opposite lateral funiculus, while in man a part of the fibers go over into the opposite lateral funiculus and a smaller part run without decussation into the homolateral anterior funiculus.

There have been published a large number of articles dealing with such variations in the position of the pyramidal tract, and with the corresponding variations in the pyramidal decussation; but very little attention has been paid to the character of the fibers of which this fasciculus is composed. A study of sections of the spinal cord of the rat, guinea-pig, rabbit and cat prepared by the puridine silver technique has brought to light great differences in the pyramidal fibers in these different animals. The variations in the size of the axons and in the degree to which the myelin sheaths are developed are no less striking, and probably more significant, than the variations in the position which the tract as a whole assumes. It is with the characteristics of these fibers in the white rat that this paper is primarily concerned and we

hope to follow it with a comparative study of the pyramidal fibers in several different orders of mammals. For this reason no attempt will be made at this time to give a comprehensive review of the literature.

Stieda ('69) noticed that the pyramidal tract in the mouse occupied the posterior funiculus. Spitzka ('86) showed that this position was characteristic for the rat and the guinea-pig. These observations were confirmed on the rat by Von Lenhossék ('89) and Bechterew ('90), using the embryological method of Fleischsig. Further confirmation was obtained through the application of the Marchi stain to the degenerating tract in the rat by Goldstein ('04), Van der Vlort ('06) and Miss King ('10).

TECHNIQUE

In this investigation the pyridine-silver (modified Cajal) technique (Ranson '12) was used as the principal method and the results were controlled by the use of the Weigert and the Pal-Weigert methods. For the Weigert methods some of the cords were fixed in Müller's fluid and others after fixation in 10 per cent formalin were mordanted either in Müller's fluid or in the following solutions:

Primary mordant

Bichromate of potassium	5.0 grams
Fluorchrom	2.0 grams
Water, ad.	100.0 cc.

Secondary mordant

Acetate of copper.....	5.0 grams
Acetic acid (35 per cent).....	5.0 grams
Fluorchrom	2.5 grams
Water, ad.....	100.0 cc.

The usual staining and differentiating solutions were employed and paraffin as well as celloidin sections were utilized. An effort was made to use as many different combinations as possible, and in this way to exclude the possibility that the characteristic staining of the pyramidal tracts was due to the particular modification of the method employed.

The pyramidal fasciculi in the white rat take a very light stain with the Weigert methods, appearing under low magnification as grayish blue areas clearly marked off from the remainder of the white substance which stains a deep blue. On the other hand, the pyridine-silver technique causes these tracts to stand out from the rest of the cord because of the dark brown color which they assume. Since the remainder of the white substance stains a very light brown, the contrast is striking and could be equalled only by the most fortunate Marchi preparations. This contrast is equally evident in the decussation and after the pyramidal tracts have assumed their position on the ventral surface of the medulla.

Since nowhere in the literature are to be found altogether satisfactory figures and descriptions of the position and shape of the pyramidal tracts at different levels of the rat's medulla and spinal cord, it seems desirable again to go over these purely topographical features before taking up the finer structure.

TOPOGRAPHY

The changes in shape, size, and position of the tract at various levels can best be described in connection with figures 1 to 7. Figure 1 was drawn from a section through the upper end of the decussation of the pyramids. At and above this level in the medulla the pyramids are situated on either side of the anterior median sulcus, but do not project ventrally as they do in the human brain. Fibers can be seen detaching themselves from the pyramids and running backwards to decussate as small bundles, or as individual fibers. On reaching the gray substance they spread out rather diffusely in the form of small branching bundles, many of whose fibers end within the medulla at the level of their decussation.

Where the decussation is at its height (fig.2) the crossing bundles are of large size. They run backward at some distance from the central canal, and are gathered together on the dorsal surface of the gray substance into two large fasciculi. These are at first some distance apart, but approach each other in the lower part of the medulla. Figure 3 represents the lowest level of the

With the exception of figures 5 and 9, which are from Pal-Weigert preparations, the drawings were made from pyridine-silver preparations. All drawings were made with a Leitz microscope.

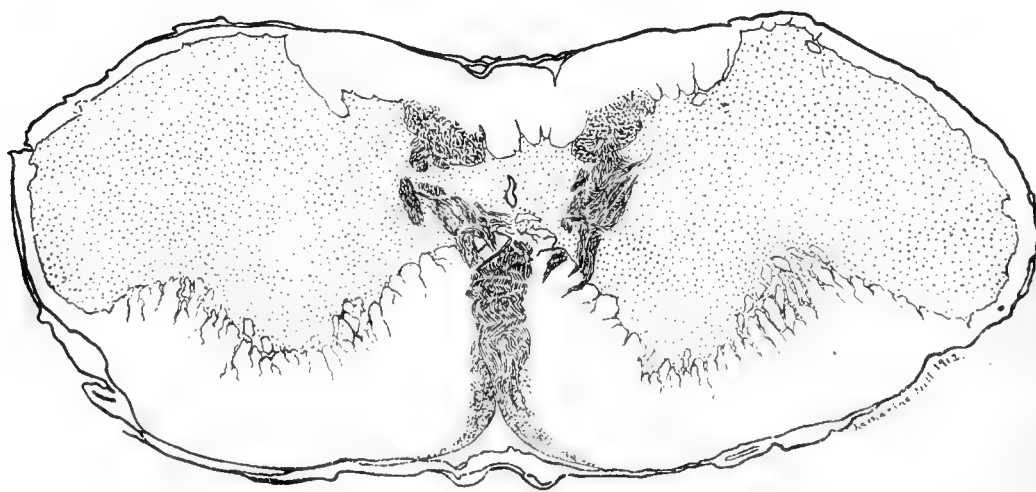
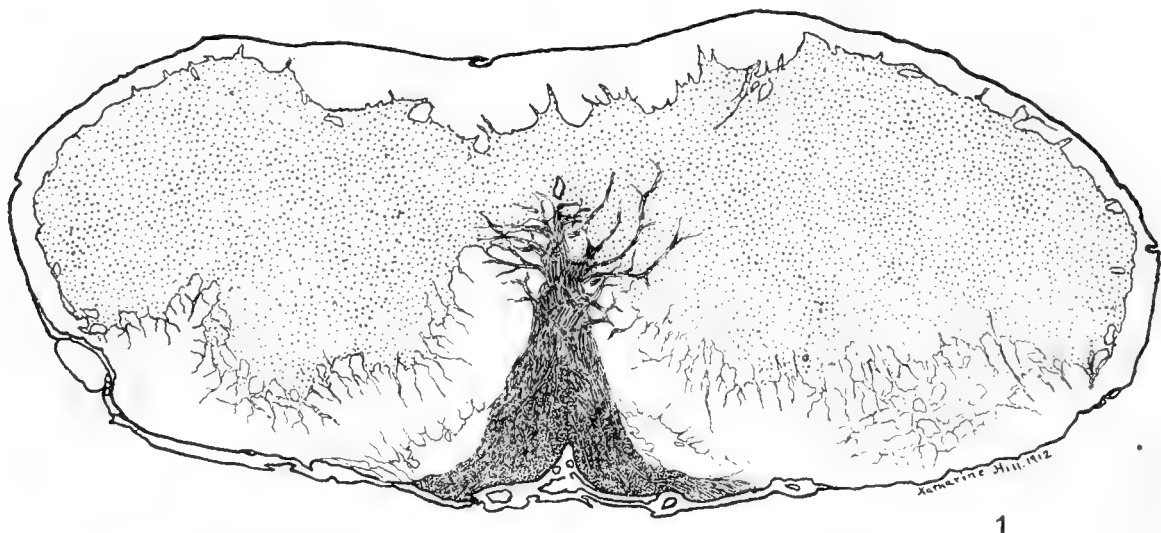


Fig. 1 Medulla oblongata, upper end of decussation of the pyramids. Ocu. 0, Obj. 3.

Fig. 2 Medulla oblongata, middle of the decussation of the pyramids. Ocu. 0, Obj. 3.

decussation. The pyramids have disappeared from the ventral surface of the medulla; at *a* some of the lowest decussating fibers are indicated. The two large pyramidal fasciculi lie near the posterior median septum.

The pyramidal decussation differs from that in man, in that the fibers go over into the posterior instead of the lateral funiculus.

and in that the decussation in the rat is complete. No pyramidal fibers run directly down on the same side into the anterior funiculus of the cord.

In the cervical region of the spinal cord (fig. 4, *c.* 7) these tracts occupy the ventral portion of the posterior funiculi and are closely

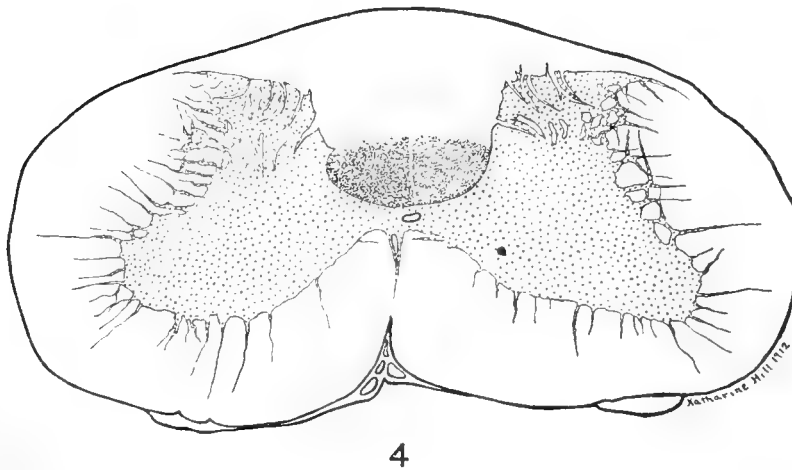
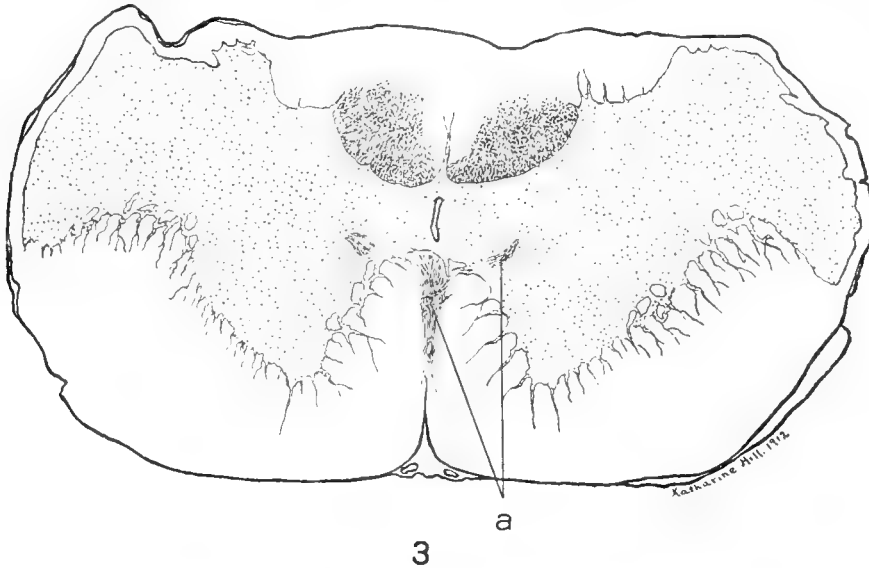


Fig. 3 Medulla oblongata, lower end of the decussation of the pyramids; *a*, lowest decussating fibers. Ocu. 0, Obj. 3.

Fig. 4 Seventh cervical segment of the spinal cord. Ocu. 0, Obj. 3.

approximated to each other and to the curved surface of the culumna posterior. Since the medial and posterior surfaces of of the bundle are almost at right angles to each other, the shape of the tract, as seen in sections through this level of the cord, is that of one-fourth of a circle. There is not as much intermingling

of the pyramidal with surrounding fibers as one sees in the human cord. In the rat the tract stands out as sharply outlined in the preparations as it is in the drawings.

In the upper thoracic region the bundle changes its shape somewhat, since the posterior surface forms an acute angle with the medial surface; and the area occupied by the tract in sections of this part of the cord has the shape of an eighth part of a circle. Figure 5 was drawn from a Pal-Weigert preparation taken at

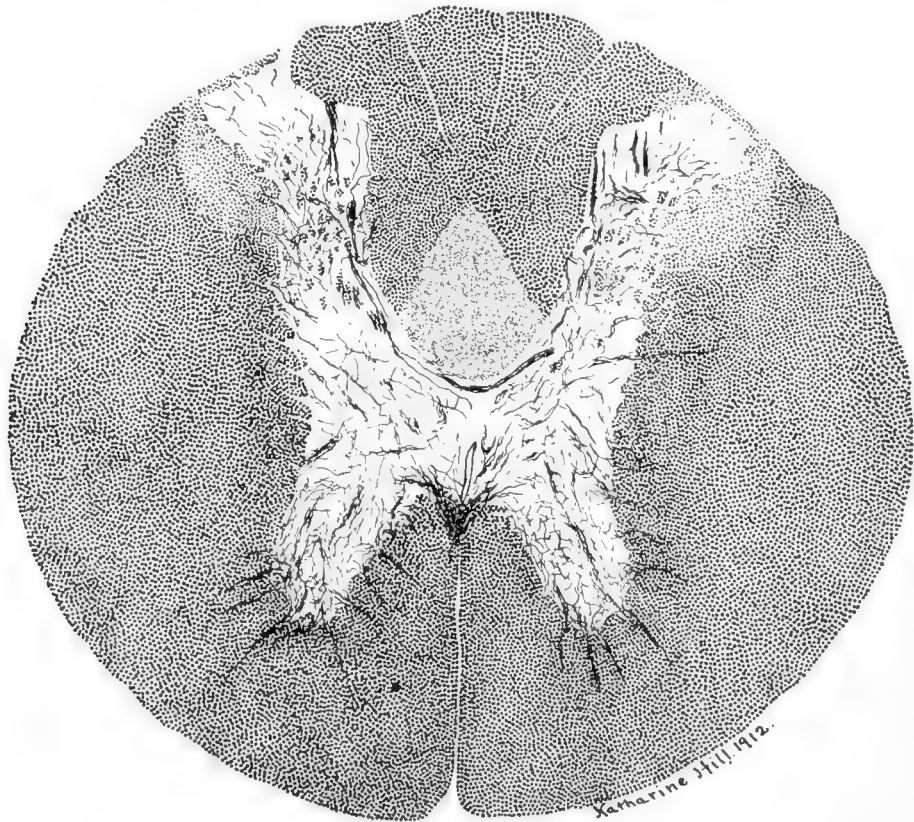
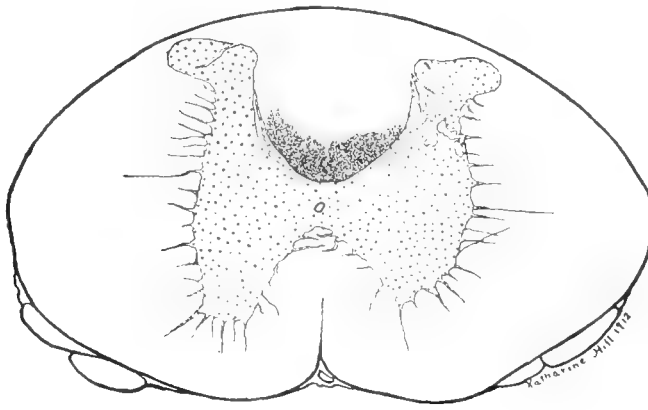


Fig. 5 Fourth thoracic segment of the spinal cord. Ocu. 3, Obj. 3.

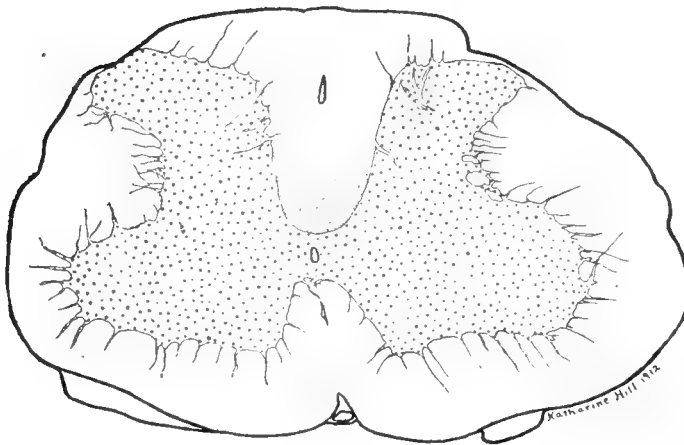
about the level of the fourth thoracic segment and shows the tract clearly outlined by its fainter staining from the remainder of the white substance.

In the mid thoracic segments the bundle becomes rounded or oval in outline, and in the lower thoracic segments (*T.12*) it spreads out laterally along the posterior surface of the gray substance (fig. 6). In the upper lumbar region the outline of the tract is no longer so sharply indicated, and the tendency to spread

out laterally is more pronounced. In the lower lumbar segments (fig. 7, *L. 5*) the fibers are diffusely scattered through the ventral part of the posterior funiculus and the tract has lost entirely its definite outline.



6



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Fig. 6 Twelfth thoracic segment of the spinal cord. Ocu. 0, Obj. 3.

Fig. 7 Fifth lumbar segment of the spinal cord. Ocu. 0, Obj. 3.

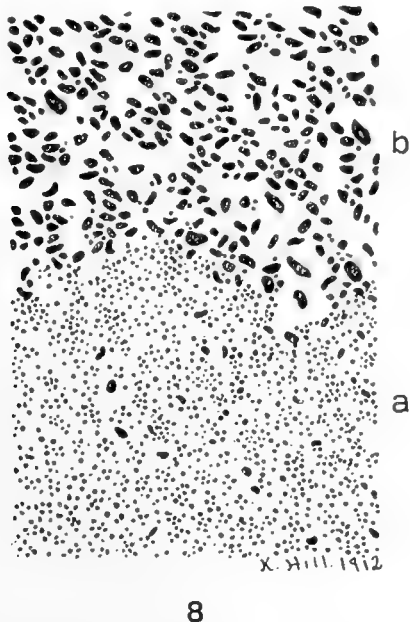
There is a progressive decrease in the size of the pyramidal tract as it runs caudad through the posterior funiculus of the spinal cord. This is seen on comparing the seventh cervical (fig. 4) with the twelfth thoracic segment (fig. 6).

STRUCTURE

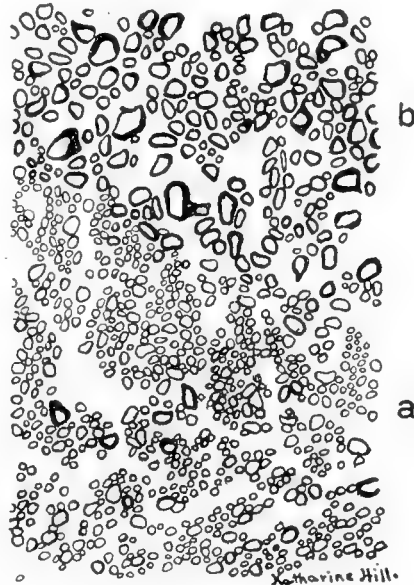
We turn now from the consideration of the tract as a whole to the characteristics of the individual fibers and shall learn why the tract stains so intensely with the pyridine-silver technique and so faintly with the Weigert methods. In pyridine-silver preparations of the spinal cord all the axons are stained; the larger ones are yellow, while the smaller ones are dark brown or black. The other elements of the white substance (such as myelin sheaths, neuroglia and blood vessels) are stained faintly or not at all. Nearly all of the axons in the pyramidal fasciculus (fig. 8, *a*) are very small and these closely packed dark brown axons give the characteristic brown color to the fasciculus as a whole. This contrasts sharply with the structure seen in the remainder of the white substance of the cord (fig. 8, *b*) where the large, light yellow axons, surrounded by thick unstained rings of myelin give rise to a lighter color and a more open structure. There are a few medium-sized axons in the pyramidal tract and a few of the very fine ones in the other fasciculi of the cord. Figures 8 and 9 were taken from the fourth thoracic segment of the spinal cord at the border of the pyramidal fasciculus.

As has been said, the pyramidal tract takes a light grayish blue stain in Weigert preparations (fig. 5). It contains many very fine medullated fibers and a few of medium size (fig. 9, *a*). The myelin sheaths of the pyramidal axons are thinner than the sheaths on axons of the same size in other regions of the cord. Some are so thin and faintly stained that they are just recognizable. The medullated fibers do not occupy all the space in the tract but are separated from each other by unstained spaces. When we compare the Weigert and the pyridine-silver preparations of the same level of the cord we see that the axons in the pyramidal fasciculus (fig. 8, *a*) are much more numerous than the myelin sheaths (fig. 9, *a*) and that the axons are more closely packed together. In any given section, therefore, many of the axons are without myelin sheaths. This is susceptible of two interpretations: either many of the pyramidal fibers are entirely non-medullated; or the myelin sheaths of the pyramidal fibers are

interrupted, medullated and non-medullated stretches succeeding each other along the course of the same fiber. In any case, the medullation of the pyramidal tract in the white rat is incomplete, and such sheaths as are present are very thin. It is not possible to draw the same sharp line between entirely non-medullated and fully medullated fibers that can be drawn in the spinal nerves.



8



9

Fig. 8 An area from the fourth thoracic segment of the spinal cord at the boundary between the pyramidal, *a*, and the cuneate, *b*, fasciculi; only axons are shown. Ocu. 3, Obj. 2 mm.

Fig. 9 An area from the fourth thoracic segment of the spinal cord at the boundary between the pyramidal, *a*, and the cuneate, *b*, fasciculi; the myelin sheaths are shown. Ocu. 3, Obj. 2 mm.

The same results are given by both the old Weigert and the Pal-Weigert methods. Care has been taken not to decolorize the smallest myelin sheaths. To avoid this, sections 8 to 10 μ thick were used, and the decolorization was stopped just short of completion, leaving a diffuse light blue tint in the background. In these thin preparations it was possible to see dark blue myelin sheaths clearly outlined against the lighter background. The number of sheaths seen in this way corresponded with the number seen in the more fully differentiated preparations. When a well differentiated preparation is stained with acid fuchsin a

counter-stain of the axons is obtained. The pyramidal tract stains intensely with the fuchsin because of the predominance of axon substance in its composition.

Non-medullated fibers are also found in other parts of the white substance of the rat's spinal cord but are much less numerous than in the pyramidal fasciculus.

It should be added that all these observations were made on well developed adult rats, and are not to be explained by an immaturity of the individual animals employed.

These observations on the character of the fibers in the pyramidal tract of the white rat were made in connection with a search for the path within the spinal cord taken by the non-medullated fibers of the dorsal roots (Ranson '12). It is conceivable that they might run into the ventral portion of the posterior funiculus and ascend in the region occupied by the pyramidal tract; and since the number of non-medullated fibers from the dorsal roots is very considerable, such ascending fibers might represent all of the non-medullated fibers seen in this part of the cord. That is to say, the tract described in the first part of this paper might be a mixed one consisting of descending medullated fibers from the motor cortex and ascending non-medullated fibers from the dorsal roots.

In order to rule out this possibility, the following experiment was performed on adult albino rats. Under aseptic precautions the sciatic nerve was exposed in the upper part of the thigh, grasped with artery forceps and torn out of the pelvis. Five experiments were made. In one case two dorsal roots and their ganglia came away with the sciatic, in the remaining four only one root and ganglion. Each animal was killed after from twenty-four to twenty-eight days. No attempt was made at the autopsy to determine which of the roots associated with the sciatic was torn away in the operation. Pyridine-silver preparations were made of the lumbosacral portion of each of these cords. A varying degree of degeneration was seen in the last lumbar segments, depending upon the amount of damage done to the ganglia and roots when the sciatic was torn out. But in each case a very definite degenerated area could be seen, from which most of the

axons had disappeared. Figure 10 illustrates the appearances seen in these sections. It will be observed that the posterior funiculus on the left side of the drawing is considerably smaller than that on the right and contains a degenerated area (a) reaching from the surface of the cord to the interval between the two pyramidal fasciculi. The area occupied by large undegenerated medullated fibers on the left side (b) is about one-half that on the right. But there is no clearly marked decrease in the size

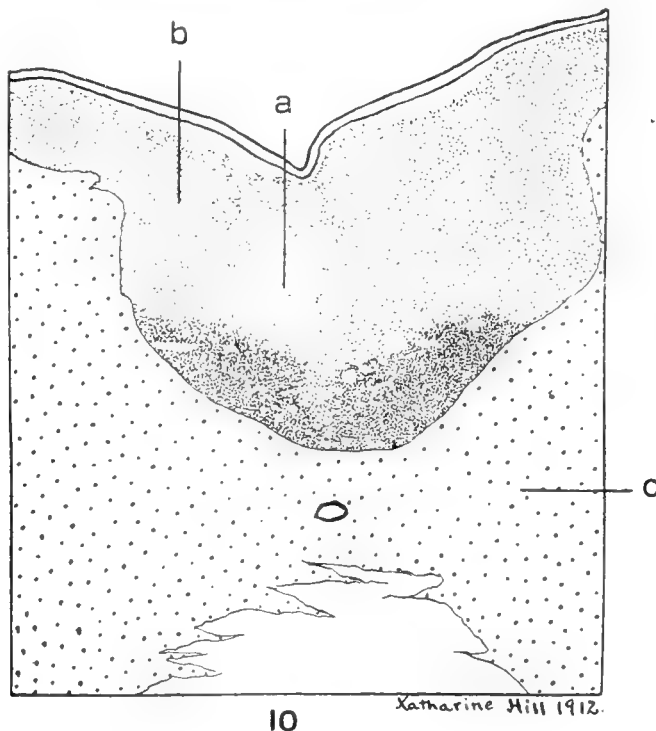


Fig 10 Part of a section through the lumbar portion of the spinal cord showing degeneration, *a*, in the cuneate fasciculus. Undegenerated portion of the cuneate fasciculus, *b*; gray substance *c*. Ocu. 3, Obj. 3.

of the pyramidal fasciculus nor any noticeable degeneration of the axons within its area.

In order to compare more accurately the areas occupied by the pyramidal fasciculi on the normal and operated sides, these areas were roughly computed in sixteen successive sections. This was done by tracing the outlines of the tracts with the camera lucida on millimeter paper, and determining the number of square millimeters covered by the areas thus projected. It was found that the tract on the operated side was 3 per cent smaller than that

on the normal side, a decrease which could be accounted for by the presence of a few dorsal root fibers within the area of the tract. Since the tract decreased so little in size and since there were no other evidences of degeneration within its territory, it is obvious that the non-medullated fibers which it contains do not belong to another system arising in the dorsal root ganglia. These experiments also serve to emphasize the sharpness with which the regions occupied by the pyramidal tracts are limited in the white rat.

Watson ('03) noticed that in the Pal-Weigert preparations of the spinal cord of the adult albino rat the pyramidal fasciculus was only slightly stained and he attributed this to a supposedly different chemical composition of the myelin in the sheaths of these fibers. Miss King ('10) states that, when compared with the Marchi preparations of the pyramidal fasciculus in the rabbit, cat and dog, the Marchi preparations of this tract in the rat reveal a striking paucity of fibers "so that in this animal the so-called primary motor path is probably only of secondary importance." In view of the incomplete medullation of the pyramidal tract in the rat it is easy to understand Miss King's results. Although there is an abundance of axons there are few well medullated fibers, such as would respond readily to the Marchi stain. Just how far the incomplete medullation of this tract is an indication of an incomplete development of its function is a matter which it would be very difficult to decide. Attention has been called to the peculiar light staining of this fasciculus in Weigert preparations of the spinal cord of animals belonging to widely separated groups. Ziehen ('99 and '00) mentions it as occurring in the pseudochirus, the sheep and the rat. Dräseke ('04) observed that in the mole the pyramidal fibers lose their myelin sheaths as they pass from the medulla into the anterior funiculus of the spinal cord, into which they go without decussation. Here they form a medially placed oval field somewhat ventral to the anterior commissure. This oval area is very faintly stained in Weigert preparations and is almost devoid of medullated fibers. Bischoff ('00) states that in the hedgehog the pyramidal fibers are so fine and possess so delicate a myelin sheath that the Marchi stain

does not give good results. He could follow only a few degenerating fibers into the spinal cord where they lay in the homolateral anterior funiculus. They could not be traced beyond the upper cervical segments. These findings seem to indicate a condition in the hedgehog similar to that found by Dräseke in the mole.

It would seem, therefore, that the incomplete medullation of the pyramidal tract in the rat is not a characteristic peculiar to these animals but is related to similar conditions in at least some marsupials (pseudochirus), some species of insectivora (mole, hedgehog), some other rodents (guinea-pig) and some ungulates (sheep). Since, however, the observations on these other animals were confined to myelin sheath stains it seems desirable to make a comparative study of the question with an axon stain. Such an investigation is now under way.

In man, medullation of the pyramidal tracts begins shortly before birth and is not completed until the second year. It is obvious that this tardy medullation is rendered more significant in the light of these facts concerning the condition of this tract in some of the lower animals.

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ON THE DEVELOPMENT OF THE MEMBRANA TECTORIA WITH REFERENCE TO ITS STRUCTURE AND ATTACHMENTS

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FOURTEEN FIGURES

For more than half a century various investigators have studied the structure of the cochlea with conflicting results. Comparatively recently Kishi ('07) and Shambaugh ('07) have championed the view originally held by Retzius ('84), that the membrana tectoria remains attached to the organ of Corti. They maintain moreover that the membrana is the logical structure through which sounds are transmitted to the auditory cells, and that it acts as a resonator. This function Von Helmholtz was the first to ascribe to the rods of Corti and later with Hensen to the fibers of the basilar membrane.

Hardesty ('08) denies the existence of an attachment between the membrana and the spiral organ, yet maintains that through the medium of the membrane sound vibrations are transmitted to the auditory hairs. A voluminous literature has been written dealing with the physiology of an organ the structure of which is inadequately known. To ascribe a definite function to the membrana tectoria we must first know with absolute certainty its structure and attachments. No physicist will accept as an important organ of hearing a membrane of indefinite structure and with no fixed position with reference to the spiral organ itself. For such a floating membrane, as we shall show later on, may readily change its position and relations to the auditory cells, and would certainly interfere with and interrupt the auditory function.

From the physiological standpoint it is then necessary to answer two anatomical questions before we may assign the membrana a logical rôle in the processes of audition: (1) Has the membrana any definite and peculiar structure which may adapt it to the transmission of sound vibrations? (2) Is the membrana so attached as to be constantly in contact with the hair cells of the spiral organ? We hold that as yet these questions have received no adequate answer.

The membrana is described by nearly all of those who have investigated it, as being an elastic cuticular structure containing within its interstices a more or less fluid matrix. This cuticular membrane has been variously interpreted as formed of agglutinated cilia or hairs (Ayers '92); as lamellar (Shambaugh '07); as a reticulum (Retzius '84); as a coagulum of the endolymph (Czinner and Hammerschlag '98); as a fibrous feltwork embedded in a gelatinous matrix (Hardesty '08); as composed of fibers and cuticular layers (Held '09).

As to its attachments there is a division of opinion, some holding that it is attached to the spiral organ (Retzius '84, Coyne et Cannieu '95, Kishi '07, Shambaugh '07) while this is denied by others—more recently by Rickenbacker ('01), Hardesty ('08) and Held ('09).

The classic figures given in textbooks of anatomy and histology (fig. 1) show it as a lamellated membrane attached to the labium vestibulare and extending outward over the internal spiral sulcus and the organ of Corti. Its outer edge thus floats free in the endolymph and the lamellae are shown parallel to the ends of the hair cells. The textbooks usually state that it takes its origin from the limbus spiralis and hence must grow by the development of new lamellae from beneath.

The conclusions of those who have worked on the development of the membrana tectoria are as contradictory as are those who have interpreted its structure. Kölliker ('61) originally described the membrana tectoria as a finely striated membrane arising from the columnar epithelial cells of the basal wall of the ductus cochlearis. Hensen ('63), Retzius ('84), Pritchard ('78), Schwalbe ('87) and others agree as to its cuticular origin. Czinner

and Hammerschlag ('98) assert that it arises independently as a coagulum or concretion of the endolymph, and later becomes attached to the epithelium. Ayers ('92) maintained that the fused hairs of the auditory cells form the membrana tectoria and that their agglutinated tips later fuse to the labium vestibulare, while Böttcher ('70) asserts that it is formed from hairs arising from the epithelial cells of the cochlear duct. Coyne et Cannieu ('95) found that the membrane was attached to the organ of Corti or had been torn away from it and that it shows a lamellar or reticular structure according as it is sectioned through the

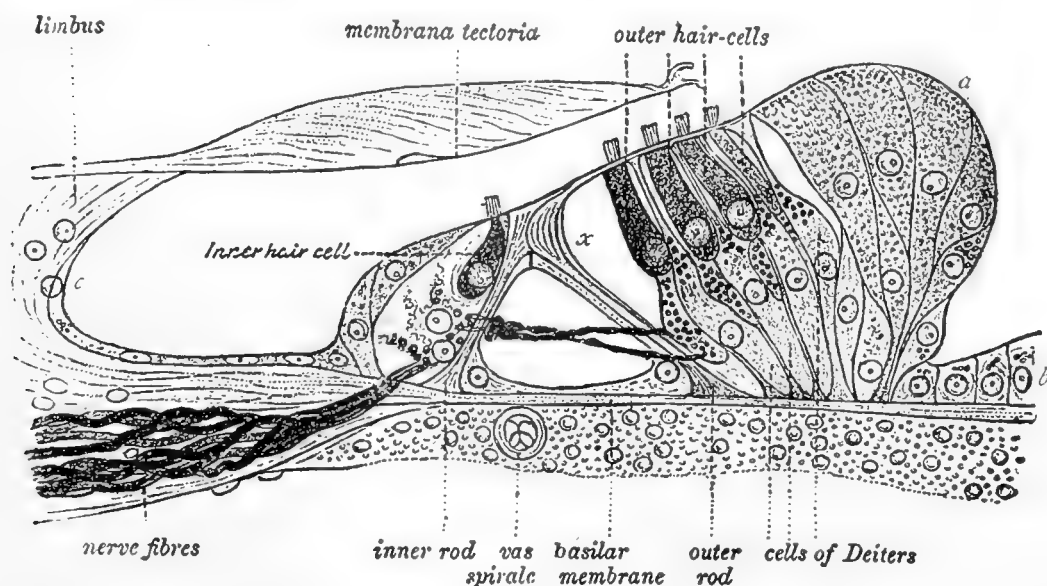


Fig. 1 Semi-diagrammatic representation of the organ of Corti and adjacent structures (Merkle-Henle).

axis (modiolus) of the cochlea or in a plane perpendicular to this. There are thickenings at the angles of the reticulum and these thickenings give to the membrane the striated appearance which is seen in other sections.

Rickenbacher ('01) after studying five stages in the development of the cochlea of the guinea-pig concludes that there are two epithelial ridges (wuelste) in the floor of the cochlear duct. The inner axial ridge is the greater and its cells give rise, first, to the major part of the membrana tectoria as a cuticular secretion which increases in thickness outwardly by the addition of new lamellae. Later from these cells are formed the epithelium

of the labium vestibulare, of the spiral sulcus and the inner portion of the spiral organ. The lesser or outer ridge gives rise to the outer portion of the membrana tectoria, and later forms the outer portion of the spiral organ. In the new-born guinea-pig the tectorial membrane loses its connection with the organ of Corti, probably owing to a dissolution of a portion of the membrane. The membrane makes its appearance before the differentiation of the organ of Corti or of the hair cells. In structure it is a cuticular reticulum which later becomes swollen, convex above and is detached from the spiral organ by the secretion of endolymph beneath it. No cilia or hairs were observed by Rickenbacher until after the differentiation of the organ of Corti, and then only the hairs of the auditory cells appeared. He does not state definitely just how the cuticular reticulum of the membrane arises nor does he account for the striated or lamellar structure which is characteristic in ordinary preparations and which he figures.

Hardesty ('08) in restudying the development of the membrana tectoria finds it first in embryos of 3 cm. as a "cuticular film of appreciable thickness and decided fibrous character." Of the two epithelial thickenings in the basal epithelium *the inner only takes part in forming the membrana tectoria, the outer giving rise only to the spiral organ (of Corti)*. "Not till pigs of about 14 cm. do any preparations show evidences of differentiation of the cells of the lesser thickening into what will become the organ of Corti," says Hardesty. He thus agrees with Rickenbacher that the membrana is quite well formed before the hairs of the auditory cells appear, thus proving false the conclusions of Ayers. As he contends that the membrana does not develop over the spiral organ (though his figure 10 does not bear out this contention) Hardesty must account for its later position over and extending to the outer side of the hair cells. This he does by maintaining that owing to the retrogression of the cells which originally fill the spiral sulcus, there is an inward displacement of the spiral organ which thus causes the outer portion of the membrana to rest above and beyond it. Just how this can take place without a shortening of the basal membrane is not stated, nor do his meas-

urements show conclusively that the pillar cells have actually approached the inner angle of the cochlea a sufficient distance to warrant the change in position of the membrana tectoria. This point will be taken up in describing our own preparations. Hardesty describes an accessory tectorial membrane lying beneath the tectorial membrane proper and extending from near its outer edge to Hensen's stripe. It is composed of two sets of fibers crossing at an acute angle. In his figures these fibers form a network with diamond shaped meshes. Hardesty does not state how this accessory tectorial membrane is developed. Hensen's stripe, a line which has been described as extending lengthwise along the underside of the tectorial membrane, Hardesty regards as due to the intercrossing ends of the fibers composing the membrane. He states that its position corresponds to the line of enclapsed phalanges of the pillars. Hence the stripe of Hensen should lie between the inner and outer hair cells. Hardesty believes that it has an embryological significance: "Hensen's stripe seems to be the expression of the period at which the retrogression of the epithelium began. It also represents the line along which the thick, outer edge of thickening was last attached and along which growth was last contributed to the membrane."

Held ('09) has made a detailed restudy of the development of the organ of Corti and the membrana tectoria in the ear of the guinea-pig, rabbit, pigeon and chick. He finds that the membrana tectoria is developed as cuticular fibers by the cells of the basal epithelium of the cochlear duct. An outer cuticular layer is first formed over the greater epithelial thickening; later growth consists in the secretion of the fibers by the cells of both thickenings, the hair cells alone taking no part in their development. Thus he holds that the membrana tectoria is developed in situ over the organ of Corti. Owing to the later elongation of its cells the organ of Corti shifts its position inward (axially) but this shifting is not extensive enough to account for the position of the membrana tectoria, which overlies and may project beyond the cells of the organ. In the adult fowl Held found that the membrana remains attached to the supporting cells of the sensory

organ, but believes that in adult mammalia its attachment to the cells of the organ of Corti is lost.

Because of these contrary and diverse conclusions which the literature in regard to the development and structure of the membrana tectoria contains, and because of its importance to the physiology of audition it was determined to make a restudy of its development in pig embryos and of its structure in man. The present paper includes my work on the development only.

METHODS

Experimenting with fixing fluids it was found that formalin and Zenker's fluid preserved the membrana well but failed to bring out sharply its cuticular structure. Osmic acid of 2 per cent and Vom Rath's osmic-picric-acetic mixture was used with success in the later stages as the fixation was good and the browning of the cuticulum by the osmic acid made its structure more clear.

In the younger stages the whole head of the embryo was fixed. In the stages approaching full term the bony labyrinth was shelled out whole, and, after the stapes had been carefully removed, was immersed in the fixative two to three days. After fixation and hardening the decalcification of the older stages was completed in 80 per cent alcohol plus 5 per cent nitric acid. They were then embedded in celloidin or paraffine and cut in planes parallel and perpendicular to the modiolus of the cochleae. Preparations were thus made from pig fetuses measuring 4, 5.5, 7.5, 8.5, 13, 15, 18.5 and 20 cm. and these were compared with sections from full term fetuses. It was found that sections mounted in balsam were not favorable for a study of the membrana because its cuticular framework is of about the same refractive index as this mounting medium. Celloidin sections were therefore mounted in water, and while only temporary preparations could be made in this way, this method was of great value in determining the structure of the membrane when unstained. With sufficiently thin sections an oil immersion objective could be employed. No special staining methods were used, the browning of osmic acid

being more effectual than any stain. Nuclei were demonstrated with haematoxylin and for counter stains eosin, orange G, and acid fuchsin were employed.

DESCRIPTION OF STAGES

Aside from the thickening of the basal epithelium, stages up to 4 mm. show no important changes.

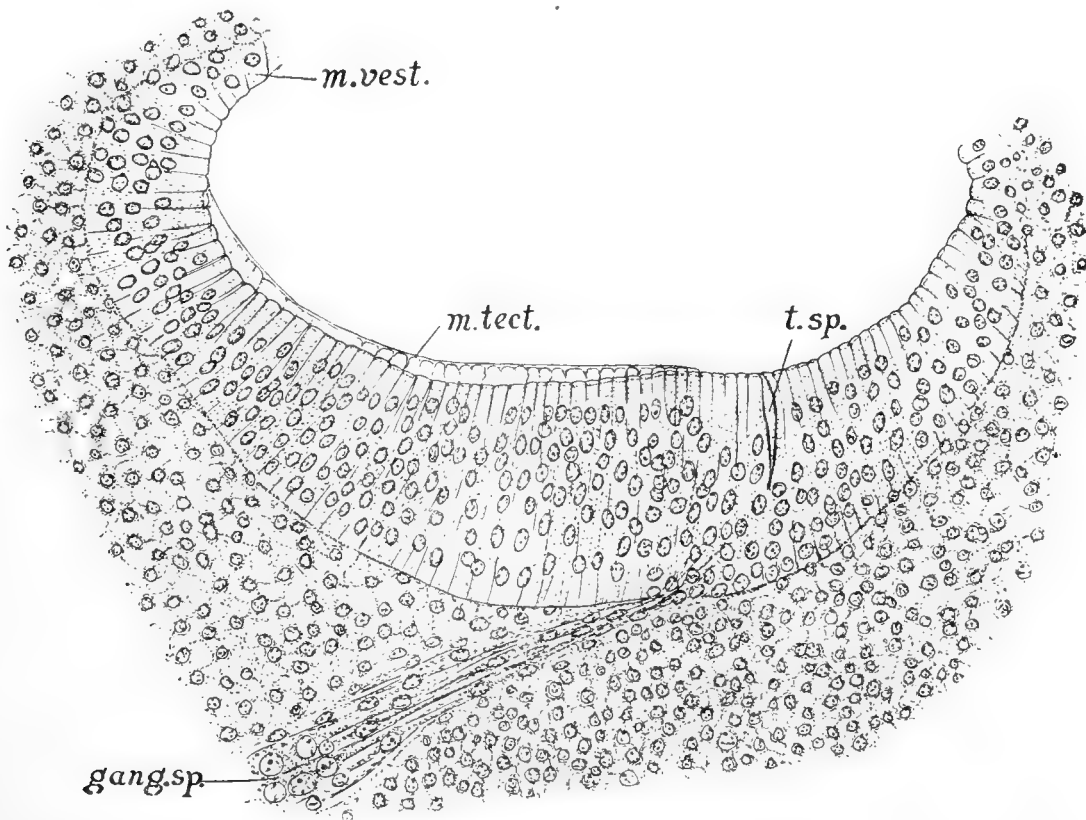


Fig. 2 Section through the second turn (fig. 13) of cochlear duct from a 5.5 cm. fetus, showing basal epithelium and origin of membrana tectoria; inner or axial side to left; *m.vest.*, membrana vestibularis; *m.tect.*, membrana tectoria; *gang.sp.*, spiral ganglion; *t.sp.*, spiral tunnel. Oc. 4, Obj. 4.

5.5 cm. stage. Here we have the basal epithelium composed of pseudo-stratified columnar cells (fig. 2).

The nuclei of the high columnar cells show a division into an inner and outer group which correspond to the greater and lesser epithelial thickenings of Rickenbacher. A section through the cochlea of a somewhat later stage shows the topography of

the spiral organ (fig. 13). The cochlear duct of the pig makes about 3.5 turns and hence a section through the modiolus shows four turns on the left and three on the right in figure 13. Figure 2 represents the basal half of the turn lettered (2) in figure 13. Of the two groups of cells which we have noted above the larger group forms the inner (axial) two-thirds of the basal wall. The nuclei of these cells are arranged in from three to six layers. They are separated from the outer group of cells by a cytoplasmic area free from nuclei. Between two cells of this area a vertical space is seen extending from the summits of the cells half way through the epithelium. This space may be due to shrinkage but represents the position of the spiral tunnel or tunnel of Corti in later stages. The outer cell group (lesser thickening of Rickenbacher) forms the outer third of the basal wall of the cochlear duct. It will eventually give rise to that part of the spiral organ which lies external to the spiral tunnel (fig. 1). From the inner epithelial cell group (greater epithelial thickening) will develop the epithelium of the labium vestibulare, of the internal spiral sulcus and that portion of the spiral organ lying internal (axial) to the tunnel. Extending over the free ends of the cells of the greater epithelial thickening may be seen a cuticular membrane which is attached between the cells by delicate threads. This cuticular membrane is the anlage of the membrana tectoria, which thus makes its appearance before the hair cells of the spiral organ are differentiated. At this stage the mesenchyma about the cochlear duct is dense and the scalae have not yet appeared. The nerve fibers of the spiral ganglion may be seen entering the epithelium internal to the organ of Corti.

8.5 cm. stage. In the second turn of the cochlea at this stage (fig. 3 and fig. 13) a space representing the spiral tunnel extends nearly through the thickness of the epithelium. The cells on each side of the tunnel are differentiating the pillars of Corti and a single inner and three outer hair cells are conspicuous. By the rapid division and elongation of the cells of the greater epithelial thickening the epithelial wall has been bent basalwards forming a concavity above and a convexity below. The concavity is the first trace of the internal spiral sulcus. The nuclei of the

greater cell thickening show not so many layers as in the previous stage but the cells are longer than the pillar cells of the spiral organ. Near the inner angle of the cochlear duct the membrana tectoria has increased very little in thickness and still forms a thin cuticular layer over the epithelial cells. Externally the membrana now extends beyond the outer hair cells of the spiral organ at which point a thin cuticle is just being formed. Over the spiral sulcus the growth of the membrana has been most rapid and here it is thickest. It appears to be composed

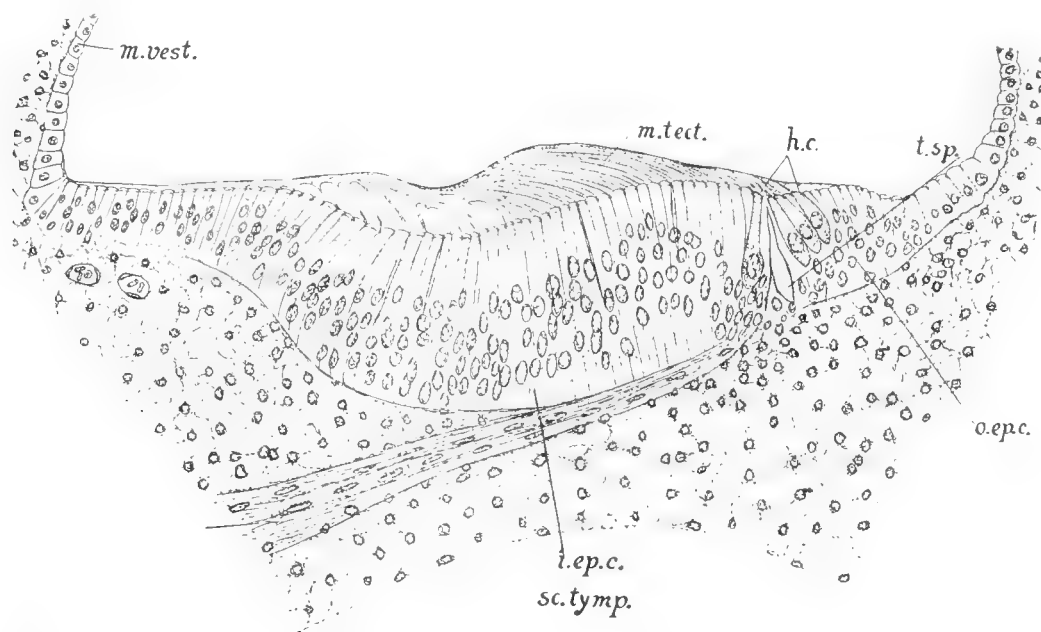


Fig. 3 Section through the second spiral of the cochlear duct from a fetus 8.5 cm. long, showing the basal half of the cochlear duct and a portion of the scala tympani; *h.c.*, hair cells of spiral organ; *i.ep.c.*, inner epithelial thickening; *o.ep.c.*, outer epithelial thickening; *sc.tymp.*, scale tympani; other lettering as in figure 2. Oc. 4, Obj. 4, t. l. 160.

of numerous parallel fibers or lamellae which are attached to the epithelium between the cells. When traced upwards away from the cells the lamellae converge and curving inwardly are continuous with the thin plate-like inner portion of the membrane which overlies the labium vestibulare.

The appearance of the tectorial membrane at this stage has been explained correctly, we believe, by Hardesty ('08). After a cuticular layer has been formed as in figure 2, the cells internal to the sulcus spiralis secrete very slowly or cease altogether. The

other cells which are forming the membrana continue to secrete actively. At the same time these cells by growth and multiplication increase the width of the basal epithelium, carrying the spiral organ outwards. Thus the distance from the inner angle of the cochlear duct to the spiral tunnel is increased. In the second spiral of the 5.5 cm. stage as in figure 2 this distance is $140\ \mu$. In the 8.5 cm. stage the same distance is about $280\ \mu$. Cells near the pillars of Corti which are secreting the membrane may thus be carried outward approximately $140\ \mu$, while that part of the membrane first formed does not grow. As the so-called 'lamellae' are secreted at the ends of the cells and the cells are shifted outward as the lamellae lengthen, naturally the bases of the lamellae will also be carried outward while their tips remain stationary. The inward trend of the lamellae from base to tip is thus satisfactorily accounted for.

It may be well to emphasize here the fact that the greater epithelial thickening gives rise not only to the epithelium of the labium vestibulare and of the spiral sulcus but also to the inner axial half of the spiral organ, including the inner supporting cells, and possibly the inner hair cells and inner pillars. This is in agreement with the results of Coyne et Cannieu ('95) and Rickenbacher ('01) Van der Stricht and Held ('09). Hardesty states that "the lesser thickening is the first indication of the differentiation of the organ of Corti while the cells of the greater give origin to the tectorial membrane . . . and the low indifferent cells lining the spiral sulcus." This misinterpretation is important as it partly accounts for his later statement that *the membrana is not derived from the cells of the spiral organ*.

The next question to decide is the true structure of the membrana tectoria. Is the membrane composed of lamellae or hairs or fibers or is it a reticulum? If it is formed at the ends of the cells just how is it developed there? These points were decided by a study of later stages, the cochleae of a 13 cm. fetus proving most favorable material. In the various cochleae which were examined it was found that differentiation begins in the basal turn and is much less advanced in the upper turns. Thus

in the 5.5 cm. stage the membrana tectoria was not yet developed in the upper turn though it had appeared in the second turn. The upper spiral of the 8.5 cm. stage was only slightly advanced in development beyond that of the basal turn of the previous stage.

13 cm. stage. The upper coil in this stage showed but little more differentiation of the tectorial membrane than figure 3. In the second spiral, however, a marked difference may be seen (fig. 4). A fibrous basement membrane is stretched beneath the epithelium, extending between the limbus spiralis and the spiral

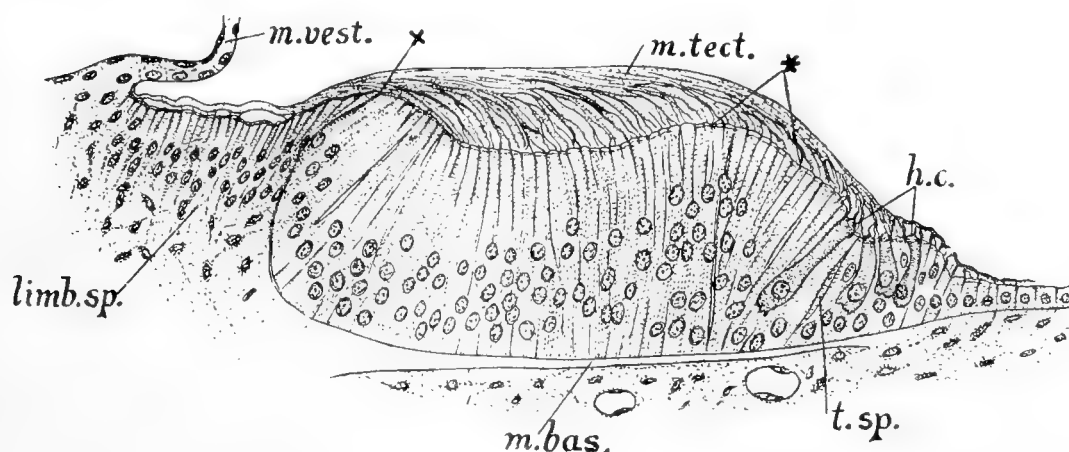


Fig. 4 Section through the basal portion of the second turn of the cochlear duct from a 13 cm. fetus; *m.bas.*, membrana basilaris; *limb.sp.*, spiral limbus. X shows point at which later labial teeth appear separating labium from the sulcus; * marks portion of field similar to that shown in figure 5. Oc. 4, Obj. 4, t. l. 190.

ligament. The epithelium itself shows a greater thickening in the region of the future sulcus spiralis, the cells being more elongate and clearer. At the axial side they show but one row of nuclei. At X these cells are sharply marked off from the epithelial cells of the labium vestibulare, the latter cells forming the so-called teeth of Huschke. The sulcus spiralis is somewhat deeper than in the preceding stage and the outer supporting cells of the spiral organ are longer and more sharply differentiated from the single layer of cubical cells external to them. The membrana tectoria is larger and extends from the inner angle of the cochlea duct to well beyond the spiral organ externally.

Over the labium vestibulare it forms a thin nearly structureless cuticular layer which becomes thicker and shows lamellae over the labial teeth. In this region it is detached from some of the epithelial cells, a condition due to shrinkage.

In the region of the future sulcus spiralis and over the inner portion of the spiral organ the membrana tectoria appears composed of delicate parallel plates which have the appearance of hairs or fibers in section, and have so been interpreted by some investigators. These plates may often be traced between the cells or about their ends. They are separated by spaces which correspond frequently to the width of the cells at the surface of the epithelium. As one follows the plates away from the epithelium the spaces become smaller and the plates or lamellae approach each other until the membrana has the appearance of a solid structure with fine parallel striations; striations which, as we have seen, converge towards the inner angle of the cochlear duct. The relation of the plates or lamellae to the cells lining the future sulcus spiralis is shown in figure 5. Using an oil immersion objective the lines were seen as sharply as in a diagram, many passing between the cells and thus taking their origin as an inter-cellular secretion. The thicker lines undoubtedly represent two plates agglutinated.

Thus far we have shown, conclusively it seems to us, that the membrana tectoria takes its origin partly from cells which in the adult line the spiral sulcus and partly from the inner supporting cells of the spiral organ; and that the cuticular plates are not like hairs or cilia in their development, as they may be traced between the cells. The next question is whether the outer cells of the spiral organ takes part in the formation of the membrana. Figure 6 shows the relation of the membrana tectoria to the cells of the spiral organ. At this stage the membrana is composed of a thin cuticular plate attached between the ends of the cells by what are apparently delicate threads. Internally (axially) the membrane is thicker and shows converging striae. Externally the membrana extends well beyond the cells of the spiral organ. The hairs of the two outer auditory cells were apparently attached to the outer surface wall of the

membrana. Except in so far as they are enclosed therein the hairs have nothing to do with the development of the tectorial membrane.

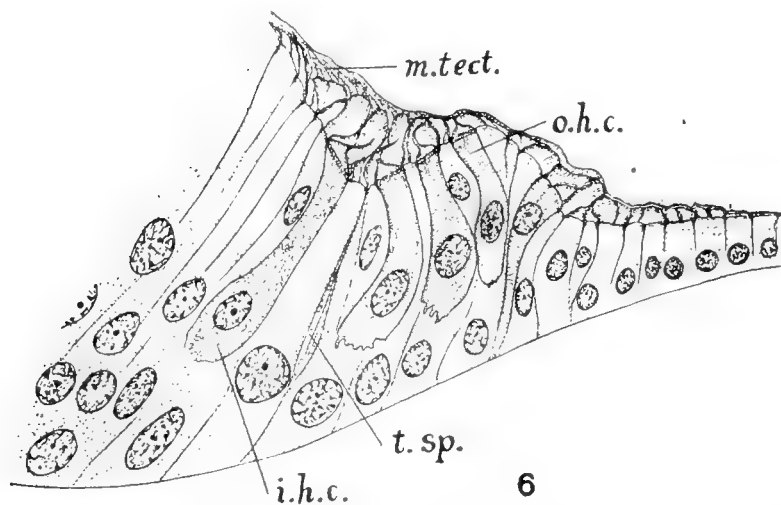
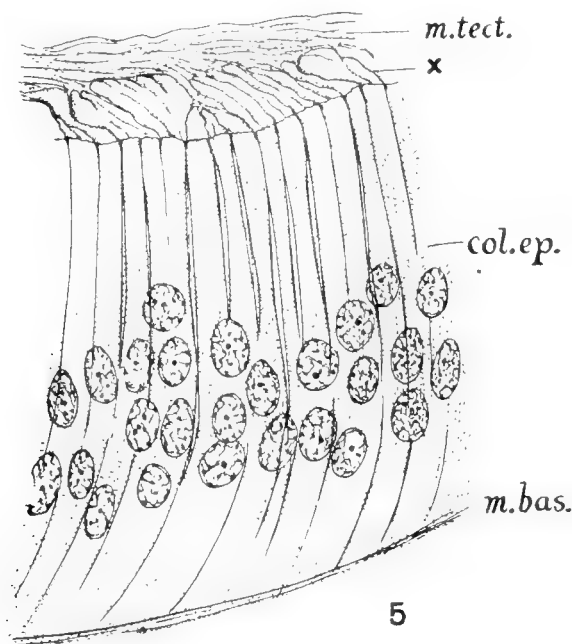


Fig. 5 Portion of basal epithelium indicated by * in figure 4, showing the structural relation of the membrana tectoria to the columnar cells; *m.tect.*, membrana tectoria; *X*, threadlike lamellae originating between two epithelial cells; *col.ep.*, columnar cells of inner group; *m.bas.*, basilar membrane. Oc. 2, 2 mm. Obj., t. l. 160.

Fig. 6 Drawing showing the membrana tectoria developing over the hair cells of the spiral organ (marked *h.c.* in fig. 4); *i.h.c.*, inner hair cell; *o.h.c.*, outer hair cell; *t.sp.*, spiral tunnel. Ob. 4, 2mm. Obj.

In describing a 14 cm. stage Hardesty says: "Up to this stage, *the membrane never overlaps the lesser thickening and in confirmation of the statement of Rickenbacher it must be said that at no stage is there good reason to assume that the cells giving rise to the organ of Corti ever have anything to do with its development.*"¹ Rickenbacher in his summary states that "Die Cortische membran ist somit doppelten Ursprungs: Die innere Zone ist die primäre welche von grossen Epithelialwulst abgeschieden wird. Die schmale Randzone ist eine sekundäre Bildung, welche an dem kleinen Epithelialwulst abgesondert wird." As Rickenbacher states that the outer portion of the spiral organ is developed from the lesser 'Epithelial-wulst' and the inner portion from a part of the greater 'Epithelialwulst,' the above quotation is not in accord with Hardesty's statement. Rickenbacher's figures (11, 12, 13) show the membrana developing over the cells of the spiral organ and attached to the hairs, so also do the figures of Held ('09) and even in Hardesty's figure 10 the membrana is shown projecting beyond the outer pillar cells and attached to the inner cells of the spiral organ. On dissecting away the membrana at this stage it was found that the thin platelike zone, overlying the spiral organ, and extending beyond it was no artifact due to coagulation, but a definite structure of the same appearance and continuous with the rest of the membrana. The total width of this membrana was found to be equal approximately to that of the membrana in the same turn of a 18.5 cm. stage.

In our descriptions we have referred heretofore to the structures composing the membrana tectoria as 'lamellae or fibers.' From the manner in which these are attached to the cells and from horizontal sections it will be seen that such terms can not rightly be applied to them. Sections of the membrana cut through the cochlea perpendicular to the modiolus or axis show their true significance. In such a section the organ of Corti (spiral organ) and the limbus are cut at right angles to the long axes of their cells, the line of section being indicated by *x* in figure

¹ Italics mine.

5. That portion of the membrana extending over the sulcus is seen cut perpendicular to the fibers or lamellae (fig. 7).

Its structure is that of a reticulum. The meshes are composed of delicate cuticular walls and at their angles are triangular or rectangular thickenings. The walls of the network are sharply defined and in unstained preparations appear highly refractive and clear. This structure can not be due to the effects of fixing reagents upon a gelatinous substance for in this case the lines of strain would not be as definite and would have a grayish, granular appearance instead of being clear and refractive as is

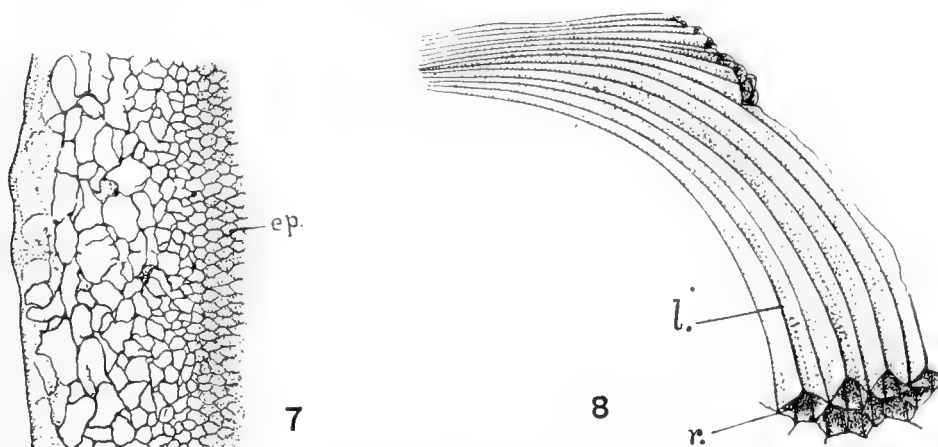


Fig. 7 Membrana tectoria sectioned in a plane perpendicular to the axis of the cochlea, thus cutting across the 'fibers.' The drawing shows its reticular structure with thickenings at the angles of the meshes. From a fetus of 15 cm.; ep., epithelium. Oc. 2, 2 mm. Obj., t. l. 160.

Fig. 8 Diagram showing structure of the membrana tectoria as proved by figures 4 to 7; r., reticulum, as seen in horizontal sections; l., 'lamellae' or 'fibers,' seen in axial sections.

the case with this cuticulum of the membrana tectoria. The spaces enclosed by the network correspond in form and size to cross sections of the epithelial cells and where the membrana approaches the epithelium may be seen to correspond to the ends of the cells. The structure of the membrane is thus neither lamellar nor reticular but 'cellular' in the sense that honeycomb is cellular. The cuticular portion of the membrane corresponds to the waxen cells and these chambers are closed during development by the ends of the epithelial cells. There is this difference in the comparison that while the 'cells' of a honeycomb are nearly

straight and of the same diameter throughout, the chambers in the membrana taper as we go from the epithelium and curve toward the inner angle of the cochlear duct and are probably irregular in length and arrangement. My conception of the structure of the membrana based upon the preparations already described is shown diagrammatically in figure 8. The reticular structure is shown at the bases of the chambers, the thickenings at the angles of the meshes extend lengthwise of these chambers and when seen in side view, as in axial sections, they give the membrane the fibrous or striated appearance which has been so frequently described. This appearance was rightly interpreted by Coyne et Cannieu ('95). In a vertical section usually more than one layer of cuticular chambers may be seen and hence the striations appear numerous, indistinct and close together. Few investigators have made horizontal sections of the cochlea and in the adult and in late fetal stages such sections are difficult to obtain. Hardesty shows a section (fig. 9) in which at *a* cross sections of the 'fibers' are seen, and he has drawn a reticulum with thickenings at the angles. He states that the fibers seem to anastomose and appear to be connected with each other by fine collateral filaments but attributes this appearance to shrinkage and coagulation. On pages 161 to 162 he states that "The membrana is not a lamellated structure. Ever since 1869 when Böttcher teased portions of it and found them to contain fibers the *fibrous structure of the membrane has been conceded by all who have studied it with reference to its structure*. Sections in different planes, as made by Coyne and Cannieu ('85) ['95] and here (Fig. 9) indicate clearly its fibrous structure."²

It is certain that Shambaugh did not concede its fibrous structure, as he states that it is lamellated. On page 132 Hardesty states: "Löwenberg ('64) thought that the membrane consisted of layers one above the other; Gottstein ('72) pictured it as structureless, and many others after these have failed to comprehend its character." Rickenbacher does not figure any very definite structure nor does he account for its development. As

² Italics mine.

to Coyne et Cannieu ('95), in describing the sections mentioned by Hardesty as confirming the fibrous character of the membrana, they say (p. 285):

Cette membrane offre l'aspect d'un réseau, dont les travées seraient constituées par une substance amorphe, claire et transparente. Ces travées circonscrivent des cavités polygonales diminuant d'épaisseur à mesure qu'on s'éloigne de l'organe de Corti pour se rapprocher de la protubérance de Huschke. Les cloisons de ces cavités se réunissent au niveau des angles du réseau et forment, en ce point, des espaisissements sur toute la longueur de leurs bords de réunion. Ces espaisissements sur des coupes radiales de la membrane se montrent sous l'aspect des stries dont nous avons déjà parlé.

Coyne et Cannieu thus are in agreement with my interpretation of the structure and on page 280 state definitely that the membrana is not composed of fibrils imbedded in a homogeneous matrix. Hardesty could not demonstrate by special stains the presence of a matrix which would hold the fibers together. His conclusions are based apparently on surface views of the membrana in which he saw an apparent fibrillar structure. The ends of the fibers which one may see on the under side of the membrana may be interpreted also as the thickenings at the angles of the reticulum shown by Coyne et Cannieu and myself and as drawn by Hardesty himself in figure 9. It is improbable that this structure can be due to shrinkage and coagulation, for the walls of the meshes are sharply defined, clear and refractive, the size of the meshes corresponds to the size of the cells in transverse section and the network may be seen attached between cells of the spiral organ when studied in serial sections.

The accessory tectorial membrane which Hardesty describes as composed of two sets of obliquely crossing fibers he figures as a reticulum with 'diamond' shaped meshes. Its probable structure is that of a reticulum and it may be explained as a thin layer of the membrana tectoria which was left adherent to the spiral organ and later was torn away. It probably represents the reticular membrane or lamina reticularis of the spiral organ which Coyne et Cannieu interpret as a portion of the membrana tectoria which has remained attached to the cells of the spiral organ. Horizontal sections also explain why the membrana, or portions

of it, have been described by some as having a reticular structure. Held ('09) figures the membrana as arising from the ends of the supporting cells of the basal epithelium in the form of parallel fibers. Yet he does not show these fibers as continuous with the cytoplasm of the cells, like the hairs of the auditory cells, nor did he study horizontal sections through the membrana.

To sum up the development of the membrana previous to fetuses of 15 cm., we may say that it is a *cuticular organ with a definite though irregularly chambered structure which is secreted between, and at the ends of the cells composing the basal epithelium of the cochlea. Both the greater and lesser epithelial thickenings take part in its development, its outer zone arising between the cells of the spiral organ. It appears first near the inner angle of the cochlea over the labium vestibulare but growth in thickness here soon ceases. Next it develops rapidly over the cells which later line the spiral sulcus and form the inner supporting cells of the spiral organ. Finally, in later stages (yet to be described), it grows rapidly over the spiral organ. From a study of my preparations it was not possible to demonstrate distinct fibers imbedded in a matrix nor are there grounds for believing that hairs or cilia take part in its development.*

18.5 cm. stage. The later stages in the development of the cochlea show the further growth of the membrana over the spiral organ, its attachment to the latter, and the metamorphosis of the high columnar cells of the inner cell group to form the lining of the spiral sulcus. We have seen in earlier stages that differentiation of the cochlear duct is much more advanced in the basal coil than in the apical. This difference is very marked in a fetus of 18.5 cm. In figure 14 the microphotograph shows sections of three turns on each side. The scalae are both large in the basal turn but in the upper turns the scala tympani is still small. The coagulated endolymph more or less completely fills the scala vestibuli. It will be seen when compared with the 13 cm. stage that the membrana has continued to grow rapidly over the spiral organ in the two upper turns but its growth has ceased and it has remained small in the basal turn. Three stages in the development of the spiral sulcus and organ

are seen. In the upper turn (fig. 14, 3 and fig. 9) the epithelial cells just external to the teeth of the labium vestibulare have become lower, free from the membrana and tend to form a simple epithelium. The space left between the membrana and the shortening cells is the spiral sulcus.

The cells remaining between the spiral sulcus and the pillars of Corti still form a very high pseudostratified epithelium. In the middle turn (fig. 14, 2) the cells lining the spiral sulcus are

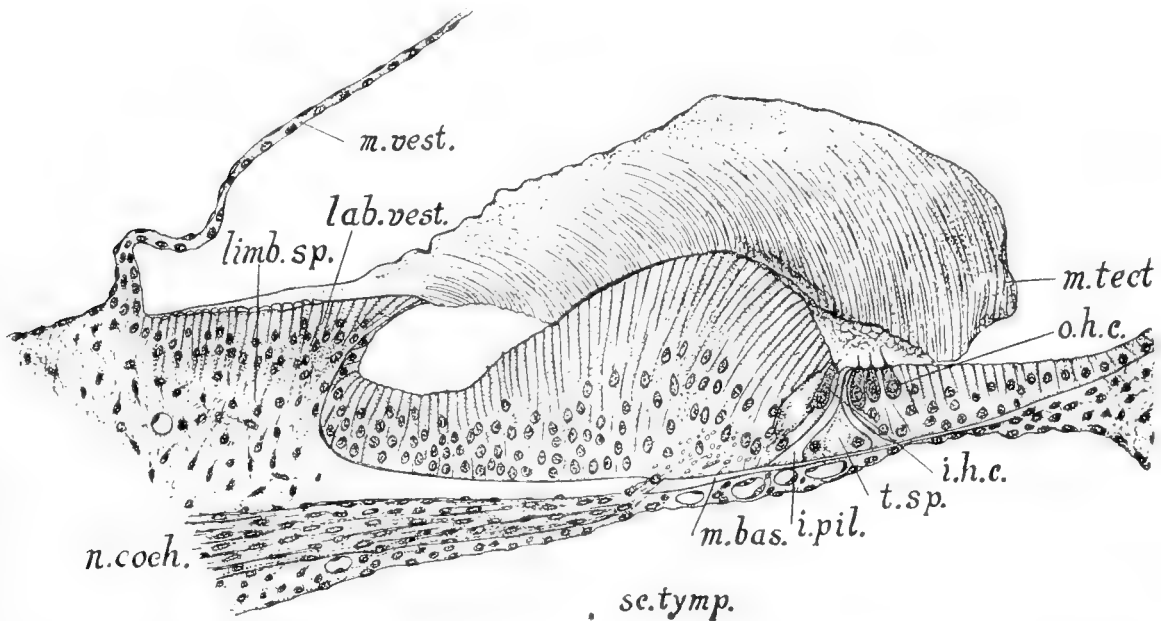


Fig. 9 Section of 3d (upper) spiral of cochlear duct of the 18.5 cm. stage; *i.h.c.*, inner hair cells; *o.h.c.*, outer hair cells; *lab.vest.*, labium vestibulare; *limb.sp.*, spiral limbus; *m.bas.*, membrana basilaris; *m.tect.*, membrana tectoria; *m.vest.*, membrana vestibularis; *n.coch.*, cochlear nerve; *sc.tymp.*, scala tympani; *t.sp.*, spiral tunnel. Oc. 4, Obj. 4, t. l. 190.

of the low columnar type with one or two rows of nuclei while the cells internal to the pillars are but little higher than the pillars themselves. Finally, in the lower turn (1) the cells lining the spiral sulcus are of the cubical type, in a single layer, and the remaining columnar cells persist as the internal supporting cells of the spiral organ. It seems probable that large numbers of the cells of the greater epithelial thickening degenerate, liquefy and disappear; those remaining flatten out and form the simple epithelium of the spiral sulcus.

In the apical turn, by comparing with figure 4 it will be seen that the membrana is but little thicker over the labium vestibulare, is much thicker over the inner cell group and thickest over the spiral organ where, in the 13 cm. stage, it was just beginning to develop. It extends far beyond the outer supporting cells. In the second turn the membrana is not so thick over the spiral organ but still extends beyond the outer hair cells. In the basal turn the membrana is thickest over the spiral sulcus and extends as far as the outer hair cells. In the two upper turns the membrana seems to be firmly attached to the cells of the greater epithelial thickening and to those of the spiral organ except along its outer zone where it shows signs of having shrunk and pulled away. In the basal turn the outer half was free but this also showed the effects of shrinkage and distortion.

Hardesty has suggested that there is a displacement of the spiral organ when the spiral sulcus is developed, thus accounting for the position of the membrana over the spiral organ in the later stages of its development.

There are a number of facts which make this hypothesis untenable: (1) Sections and dissections of the 13 to 15 cm. stages show that the membrana is developed over the cells of the spiral organ. (2) In the 18.5 cm. stage the membrana projects further beyond the spiral organ in the apical turn where the differentiation of the spiral sulcus has only just begun, and least in the lower turn where the spiral sulcus is fully developed. (3) The distance of the pillar cells from the inner angle of the cochlea (the only definite points which may be taken for comparative measurements) is about the same in the 13 cm. and 18.5 cm. stages. (4) The total width of the thickened portion of the membrana is about one-fourth greater in the 18.5 cm. stage than in the 13 cm. showing that growth has taken place along its outer border. This growth must have been supplied by the cells of the spiral organ. (5) To show that displacement takes place Hardesty measured the floor of the spiral sulcus and compared with the width of the inner cell group. There are no definite points which may be taken for measuring the floor of

the spiral sulcus, and in measuring the width of the inner cell group one is including cells which form part of the spiral organ. No accurate comparison can thus be made. The distance between the inner angle of the cochlea and the pillar cells, two definite points, may be measured with considerable accuracy and shows no important change in the position of the spiral organ from the 13 cm. to the 18.5 cm. stage, nor later in the new-born animal. (6) As the basal membrane does not shorten, the displacement theory must assume that dead passive structures like the pillars actively move inward over the surface of the basal membrane.

One argument which Hardesty uses to prove that inward displacement of the spiral organ has occurred is that the 'fibers' of the membrana when traced from its upper and outer border curve outward, downward, and then inward as though they had been pulled inward by the migration of the spiral organ. This inward curvature of the fibers is only found in the upper turns of the cochlea where the membrana is of greatest width and thickness. It may easily be accounted for. In the stages up to 16 cm. the cells of the spiral organ slant outward but as the width of the basal membrane is rapidly increasing the inclination of the chambers, hence of the 'fibers' is downward and outward. When the membrane begins to develop actively over the spiral organ the basal epithelium has attained its maximum width but as the cells are directed outward the inclination of the chambers will now be inward. When the spiral sulcus is developed by the degeneration of its cells, the outer cells of the spiral organ elongate and straighten somewhat so that they are no longer directed outward. This shifting, which is relatively slight and not enough to account for the displacement of the membrana, would nevertheless increase the inward trend of its chambers. My observations are supported by those of Held ('09) on the guinea-pig and rabbit.

In taking measurements of the 18.5 cm. stage the marked changes found are: (1) The increase in thickness of the membrana tectoria; (2) The increased distance from the inner angle of the cochlea to the labial teeth. The outer cells of the labium have

grown rapidly outward beneath the membrana thus pushing the ends of its chambers outward. The result is that in this region the chambers come to lie parallel to the surface of the labium and give the membrana a lamellated appearance which is especially marked in the lower turns of the cochlea. The membrana may be divided into zones at this stage: (1) A thin structureless zone over the inner portion of the labium vestibulare; (2) A thicker second zone of flattened horizontal chambers over the outer portion of the labium vestibulare; (3) A still thicker third zone of chambers curving downward and outward unattached over the spiral sulcus; (4) An outer zone, thickest in the upper turns with chambers trending downward, outward then inward, largely attached to the cells of the spiral organ and probably normally wholly thus attached.

The sections of the 18.5 cm. stage thus show that the membrana tectoria has developed rapidly over the spiral organ especially in the upper turns of the cochlea; that the membrana is attached to the cells of the spiral organ in the upper coils and shows shrinkage and distortion in the lower; that the inner cells of the greater epithelial thickening degenerate or persist as the lining of the spiral sulcus while the outer cells of this group form the inner supporting cells of the spiral organ. Finally there is no evidence of an inward shifting of the spiral organ sufficient to account for the position of the membrana at this stage assuming (which we do not) that it is not developed from the spiral organ and that there is a necessity for such a displacement.

The development of the structures arising from the basal epithelium of the cochlea is practically complete at 18.5 cm., but a number of cochleae were studied from the full-term fetus. The structure of the membrana at this stage has been figured by Shambaugh ('07) and Hardesty ('08) both of whom found attachments between the cells of the spiral organ and the membrana. These attachments are regarded as normal by many investigators, as due to coagulation and shrinkage by others. There is shown in figure 10 one of the many cases which occurred in my preparations showing attachment to the outer supporting cells.

The membrana is undeniably shrunken and partly pulled away from the spiral organ, but the hairs of the outer auditory cells are firmly imbedded in the membrana. The under surface of the membrana shows a thickening, *st.H.*, which according to Shambaugh ('08) corresponds to Hensen's stripe and represents the inner line of its attachment to the inner supporting cells. In other sections the membrana was firmly attached to the inner supporting cells as well as to the hairs. In all of my preparations at full-term the membrana was badly shrunken. Frequently

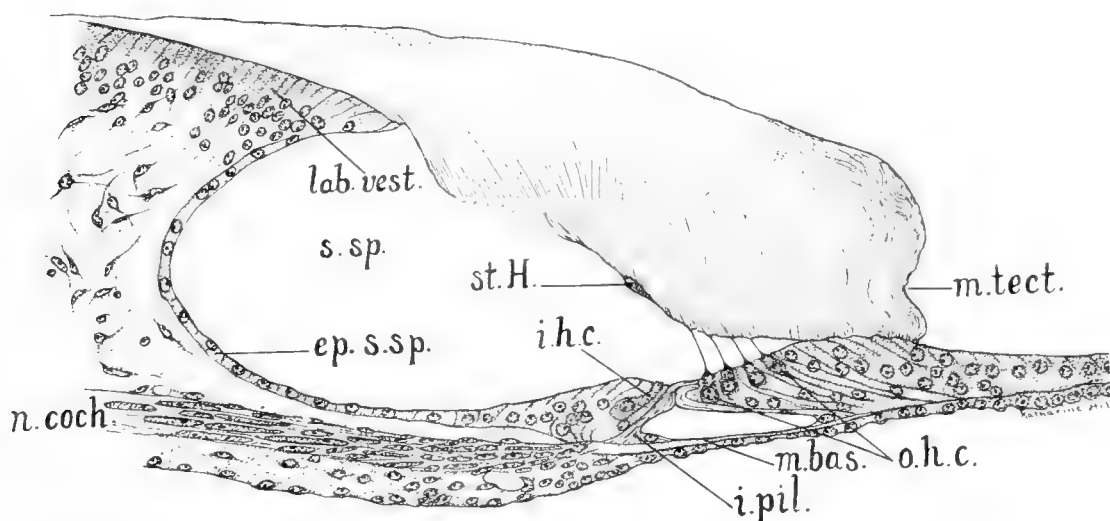


Fig. 10 Section through the apical turn of the cochlea at about full term, showing outer auditory hairs imbedded in the membrana tectoria; *ep.s.sp.*, epithelium of spiral sulcus; *i.h.c.*, inner hair cells; *i.pil.*, inner pillar; *m.bas.*, basal membrane; *m.tect.*, membrana tectoria; *lab.vest.*, labium vestibulare; *limb.sp.*, limbus spiralis; *n.coch.*, cochlear nerve; *o.h.c.*, outer hair cell; *sc.tymp.*, scala tympani; *s.sp.*, sulcus spiralis. Oc. 4, Obj. 4, t. l. 190.

also the organ of Corti was distorted, being pulled inward by the attached membrana.

The attachment of the membrana to the spiral organ I regard as normal for the following reasons:

1. In development the membrana is normally so attached.
2. The auditory hairs when attached could be traced into the membrana, even though the latter was badly shrunken.
3. The shrinking membrane frequently exerts such a pull upon the spiral organ as to distort it.

4. Physiologically and anatomically it is the condition we should logically expect if the membrana is functional in transmitting sound waves to the auditory hairs.

5. Were the membrana merely floating in contact with the hairs and unattached to them or their supporting cells it would not retain its position constantly and would thus interfere with the auditory function.

In describing the structures of the cochlea the apex is regarded as above, the base as below and the membrana tectoria as lying



Fig. 11 Dissection of the head of a pig fetus to show the position of the brain and cochlea. $\times 4$.

over the spiral organ. As a matter of fact when in its normal position the apex of the cochlea is directed cephalad and ventrad. This may be well seen in a dissection of the brain and cochlea of the pig (fig. 11).

When the pig's snout is directed downward, as in feeding, the base of the cochlea would be above, the apex below. The same would be true of the human cochlea when the head is bent forward. *The membrana tectoria would then be beneath the spiral organ* and as it is slightly heavier than the endolymph and very

flexible it would naturally sink downward and away from the spiral organ assuming that it was not attached to the cells of the latter. This would be all the more apt to occur when the membrana is subjected to the heavy jars incident to active movements, running and jumping, and should interfere with hearing. As we know, such interference does not occur.

The arguments raised by Hardesty against any normal attachment of the membrana, save to the labium vestibulare, are:

1. In dissecting the fresh membranous labyrinth to expose the membrana its outer portion could be seen floating free along its entire extent.

2. In the majority of sections it is entirely free from the spiral organ and when attached such attachments are filamentous and may be explained as abrasions of the under surface or coagulations of precipitated albumins.

3. "From the process of its development it seems probable that the membrane is free from the underlying structures," and as its outer zone acquires its position over the spiral organ by displacement, one must assume that any attachment which exists between the membrana and the spiral organ must have developed secondarily.

Hardesty's first argument bears little weight because in describing his method of studying the fresh membrana tectoria he states that it was necessary to crush the bony labyrinth with a hammer and that the disturbances caused by his dissection caused the membrana to float free from the labium vestibulare an attachment which is never entirely ruptured in carefully fixed sections. A method which would destroy the strong attachment to the labium would certainly set free the more delicate attachments to the spiral organ.

Moreover, dissections which were made by using more favorable methods did not seem to support Hardesty's observations. As to the attachments seen in sections being artifacts it is sufficient to say that I have traced the hairs into the membrana in many cases and that attachments to the inner supporting cells and to the outer hair cells are so strong as to distort the spiral organ during the shrinkage of the membrana. The very fact that the

membrana shrinks shows that its normal position has been disturbed. We may as logically assume that it was attached and in many cases has shrunken away as to assume that it floated just parallel to the surface of the spiral organ and has become pressed down upon and attached to it by coagulations.

We may assume this even more logically for *we hold, and our preparations and dissections and the observations of Held ('09) prove absolutely that the membrana is attached to the epithelial structures of the spiral organ in late fetal stages.* There is no necessity for, and my preparations afford no proof of, an inward shifting of the spiral organ and a consequent displacement of the membrana. It is therefore unnecessary to assume with Hardesty and Von Ebner ('02) that any attachment between membrana and spiral organ must be of secondary development.

While these arguments against the existence of an attachment between the membrana and spiral organ may be readily answered it is none the less true that a complete attachment to the cells of the spiral organ, such as exists in the fetus, has never been demonstrated in the adult organ. Nor, to my knowledge, has it been explained why the membrana should detach itself so readily from the spiral organ yet always retain its attachment to the labium vestibulare. First, as to the reason the complete attachment may be demonstrated in the early fetus and not in the adult: This is probably because the attachment is more firm in the fetus and because the basal epithelium and the basal membrane are less rigid in the fetus and tend to shrink *pari passu* with the membrana. In the adult or even the new-born young the tissues are less watery, more rigid and more resistant to reagents. The basilar membrane is attached to the bony labyrinth, now strongly ossified. The membrana alone shrinks to any great extent and as a result is more or less completely torn away.

Why the membrana should always lose its connection with the spiral organ and not its attachment to the labium vestibulare is explained by its structure. Over the labium it is an almost solid cuticular structure and the few chambers in this region are flattened and contain little fluid. Over the spiral sulcus the mem-

brana is composed of chambers, filled with fluid and open at their lower ends, while over the spiral organ these ends are assumed to be closed by the ends of the epithelial cells. The action of most fixing reagents and alcohol is to take water from the membrana. This would cause the open chambers to shrink, narrow, and so suddenly diminish the width and length of the membrana. As the membrana has the form of a spiral the shrinkage of the outer portion of the membrana throughout its whole length *would tend to draw it toward the labium and away from the spiral organ*, as it would diminish the diameter of the spiral. The effect would be most marked in the larger basal turns and it is there that the membrana is almost invariably torn away from the spiral organ even in late fetal stages. This alone would account for the detachment of the membrana from the spiral organ in most fixed preparations. Over the spiral organ, assuming that the membrana is attached, the chambers would be closed by the ends of the epithelial cells. Upon the action of fixing reagents or alcohol, the withdrawal of water must take place chiefly about the ends of the chambers as their cuticular walls are not permeable. The result would be the shrinkage of the chambers and their separation from the cells. Even after the membrana is freed from its attachments Hardesty has shown that it shrinks very badly during the process of dehydration and clearing, and I have noted the same. The shrinkage of the membrana may also be aided by normal tension in pulling the membrane away from the spiral organ. It is very possible that such tension exists especially in the lower turns of the cochlea, as held by Kishi ('07).

THE FUNCTION OF THE MEMBRANA TECTORIA

It is not my intention here to go into a detailed account of the physiology of audition but simply to emphasize certain anatomical facts which have a bearing upon the transmission of the sound waves. Recent investigators all agree that the hair cells form the perceptive end organ of the cochlea and that the tectorial membrane is probably the medium through which the sound waves are transmitted to the hairs of the auditory cells. Arguments against

the old theory which regarded the basilar membrane as a resonator are many:

1. The structure of the basilar membrane, clothed as it is by several layers of cells, precludes its responding to delicate stimuli (Von Ebner '02).

2. In the basal coil it is thick and rigid or may be replaced by a plate of bone though in this region the spiral organ is normally developed (Shambaugh '07).

3. Hardesty has shown that the basilar membrane is merely a flattened tendon, the fiber bundles of which are closely bound together and thus could not vibrate separately.

4. The pillars are also rigidly united, and it is probable that the functions of the basilar membrane and of the pillars in conjunction with the lamina reticularis is to give rigidity to the auditory cells in order that their hairs may respond more readily to sound vibrations.

5. The inner pillars do not always rest upon the basilar membrane but upon the edge of the labium tympanicum (Shambaugh, Hardesty).

6. Sound waves entering the perilymph would affect the basilar membrane more strongly from the side of the scala tympani yet to do this would have to pass up and down the entire length of the spiral. The amplitude of the vibrations would be lessened by this and there would be interference between the waves going up in the scala vestibuli and the waves descending in the scala tympani.

The objections raised against the basilar membrane do not apply to membrana, and there are many points in its favor:

1. The membrana is an exceedingly delicate, chambered cuticular membrane, flexible yet elastic and of a specific gravity only slightly greater than that of the endolymph.

2. It is co-extensive with the spiral organ while the basilar membrane is not.

3. It lies on that side of the spiral organ at which sound waves would first enter the cochlea by way of the scala vestibuli.

4. It is attached along its inner edge to the labium vestibulare, stretches over the spiral sulcus and overlies the spiral organ in contact with and probably attached to its cells.

5. It is narrow and thin in the basal coil and becomes wider and thicker as it approaches the apex. Measurements of the functional zone of the membrana taken from its outer border to the labial teeth, show that the sectional area of the membrana in the apical turn is from thirty to forty times that in the basal turn. Owing to the shrinkage of the membrane such measurements can be only approximate. Figure 12 shows the relative size of the membrana as seen in sections of the first, second, third and fourth turns.

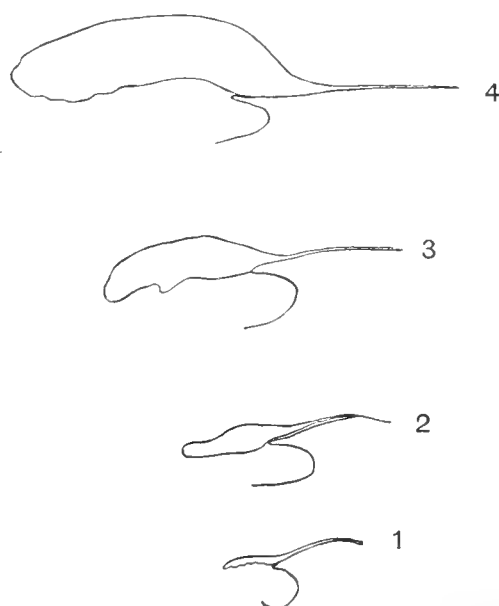


Fig. 12 Sections through the membrana tectoria of a full term fetus showing its relative size in the four turns.

From Hardesty's measurements of fresh and fixed preparations he found that the greatest width (first or apical turn) was about five times that of the minimal width (tip of basal turn) while the thickness in these same turns was as 6 : 1.

The membrana tectoria is thus to be regarded as a spiral cuticular band of delicate chambered structure, which becomes gradually thicker and wider from base to apex. This band is attached by its flattened inner edge to the labium vestibulare, spans the spiral sulcus and its outer portion is so attached between the cells of the spiral organ that the ends of the cells close the chambers and the auditory hairs project into them. It may be

that the membrana is stretched with some tension between the labium and the spiral organ, the tension being greater at the base of the spiral. Whether a membrane of such structure and attachments may act as a whole or as a resonator, must be left to physicists to decide. There seems to be little doubt, however, that the membrana tectoria is the structure through which sound waves are transmitted to the auditory cells, and that it is in every way better adapted to this function than the basilar membrane. As it is thin, narrow and perhaps under tension in the basal turns and it has been shown that notes of high pitch are perceived here, it is probable that the membrana of the basal turns responds only to the sound waves of greatest frequency. While the apical turn, which receives notes of low pitch would respond only to waves of low frequency. It does not seem possible that any cuticular chamber could alone respond sympathetically to a given note but rather that a portion of the membrana, of nearly the same breadth and thickness, vibrates as a whole.

SUMMARY

1. The membrana arises as a thin cuticular plate which is *first* developed over the free ends of the columnar cells which form the greater (inner) epithelial thickening of the basal cochlear wall.

2. As it is present in fetuses of 5 cm., before the development of the hair cells in the spiral organ, it cannot be regarded as developed from these hairs.

3. The greater epithelial thickening gives rise to the epithelium of the labium vestibulare, to the lining of the spiral sulcus and to the inner half of the spiral organ (inner supporting cells, and probably to the inner hair cells and inner pillars). The lesser epithelial thickening forms the external portion of the spiral organ.

4. The membrana grows in thickness by the secretion of a cuticulum formed between the ends of the epithelial cells, rapidly at first over the cells of the greater epithelial thickening (5 to 13 cm. stages), later over the cells of the lesser epithelial thickening.

5. In sections through the axis of the cochlea the membrana has a striated or lamellated appearance. The striae curve outward and downward from the labium vestibulare where the membrane remains thin. In sections perpendicular to the lamellae the structures of the membrana is that of a reticulum with thickenings at the angles of the meshes. It is therefore neither lamellar nor reticular but a chambered structure or 'honeycomb' of hollow tapering cuticular tubes or chambers normally filled with a fluid resembling the endolymph. The bases of chambers during development rest between the ends of the epithelial cells.

6. The thickenings at the angles of the meshes of the reticulum extend lengthwise along the whole extent of the tubes or chambers and in sections through the axis give the membrane its striated appearance, the striae having been variously interpreted as hairs, cilia, fibers and lamellae.

7. As the basal epithelium increases its width its cells are carried outward, away from the modiolus. This carries the bases of the growing cuticular chambers outward also, though their tips remain stationary. The result is the inward inclination of the chambers as they are followed from base to tip.

8. The chambered structure of the membrana explains the 'border-plexus' of Löwenberg, the accessory tectorial membrane observed by Hardesty, and the 'reticular structure' of the membrana described by various investigators.

9. In fetuses of 18.5 cm., the membrana in the upper turns of the cochlea projects outward beyond the spiral organ and is firmly attached to the cells of both the spiral organ and of the greater epithelial thickening. In this turn the spiral sulcus has not yet fully formed and the distance from the inner angle of the cochlea to the pillars is fully as great as in the preceding stage. Thus the position of the membrana cannot be ascribed to an inward shifting of the spiral organ, but is due to its rapid development from the cells of the spiral organ.

10. The attachment of the membrana to the spiral organ was proved not only by sections but by dissections of both fresh and fixed cochleae.

11. Between stages of 15 and 25 cm., the inner cells of the greater epithelial thickening change from a high pseudostratified columnar type to that of a simple cubical epithelium. These cells lose their attachments to the membrana and the space which as high columnar cells they occupied, becomes the spiral sulcus. The change is brought about by the degeneration of many of the cells and the transformation of those remaining.

12. In sections of the cochlea at full term the membrana was found attached to the inner supporting cells of the spiral organ and to the outer hair cells as well as to the labium vestibulare. This attachment is regarded as normal because it was indicated by dissection of fresh cochleae; because in development it is so attached; because the attached membrane when shrinking under the action of reagents exerts such a pull upon the spiral organ as to distort it. Lastly, because physiologically and anatomically it is the condition which we should expect to find if the membrana is functional in transmitting sound waves to the auditory hairs.

13. Although usually described as lying above, the normal position of the membrana may be directly beneath the spiral organ. As it is slightly heavier than the endolymph if unattached it would float free especially when actively moved or jarred. This would interfere seriously with the function of the organ.

14. Reasons why the membrana detaches itself from the spiral organ more readily than from the labium vestibulare are as follows: (a) The outer portion of the membrana being chambered shrinks much more than the inner zone which is a solid cuticular plate; (b) Shrinkage of the outer zone affects not only the width but the length of the membrana; (c) Being a spiral structure, the decrease in length decreases the diameter of the turns thus drawing the membrana inward. This would tend to separate it from its outer attachment to the cells of the spiral organ.

15. The arguments against regarding the basilar membrane as a medium for transmitting sound waves to the hair cells, do not hold for the membrana tectoria.

16. The membrana tectoria is a delicate chambered cuticular structure, co-extensive with the spiral organ. It is attached by its inner zone to the labium vestibulare by its outer zone between

the cells of the spiral organ thus bridging over the spiral sulcus. Its sectional area at base and at apex is as 1 : 40 approximately. As the hairs of the auditory cells project directly into the chambers of the membrana, vibrations of the membrana would be directly transmitted to them.

17. As the membrana is much thinner and narrower in the basal turns than in the apical region it is probable that different portions of it respond to sounds of different pitch. In this sense it may act as a resonator.

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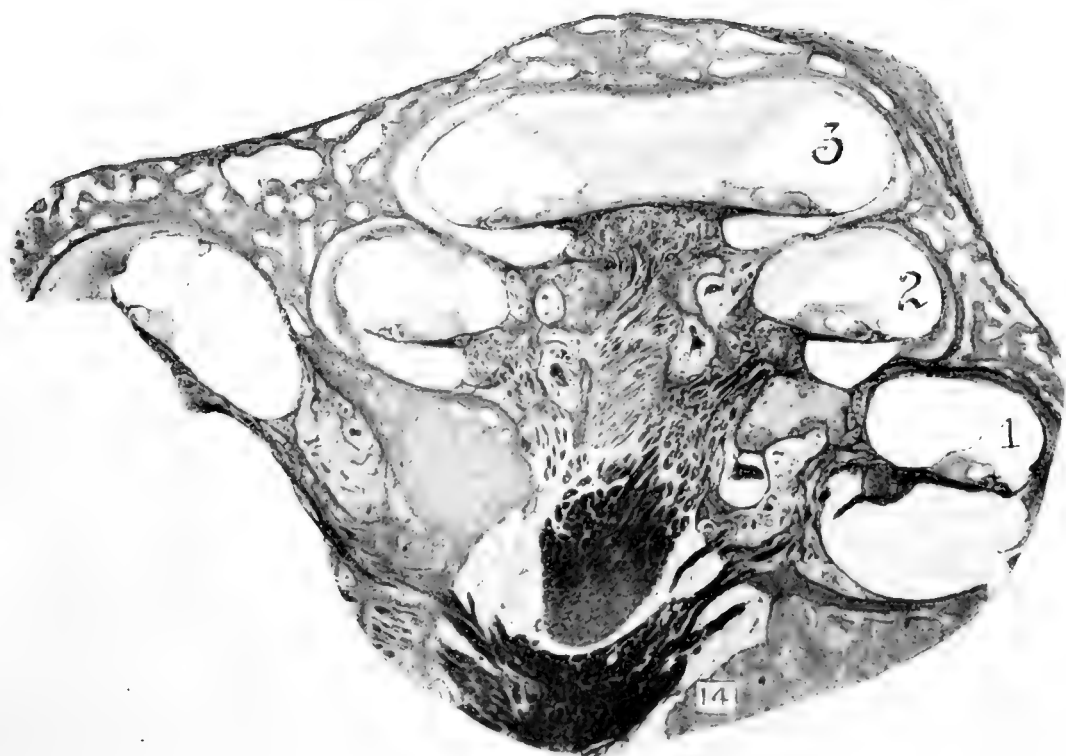
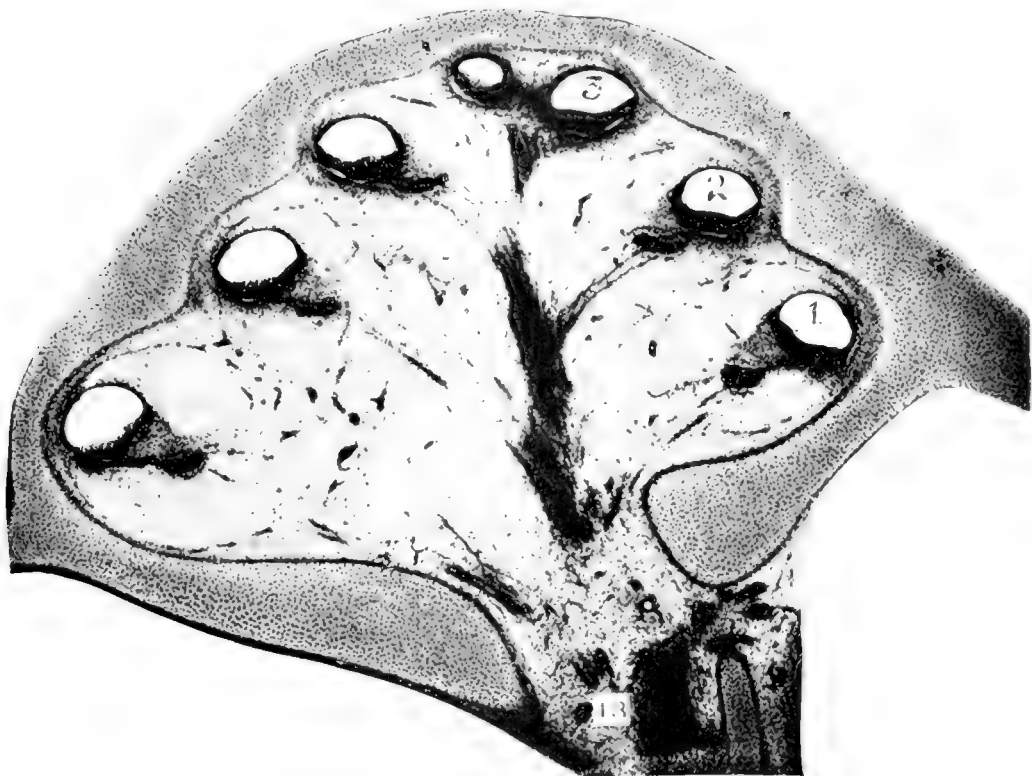
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PLATE 1

EXPLANATION OF FIGURES

Fig. 13 Microphotograph of an axial section through the cochlea of a 7.5 cm. fetus. The numerals 1, 2, 3, indicate the turns of the spiral corresponding with those similarly numbered in figure 14. $\times 20$.

Fig. 14 Microphotograph of a section through the modiolus of a cochlea from an 18.5 cm. fetus. Turns of cochlea numbered on right as in figure 13. $\times 20$.



CHROMOSOMES IN MAN

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TEN FIGURES

INTRODUCTION

The literature dealing with the subject presents a wide range of possibilities in reaching conclusions regarding the nature and number of the chromosomes of man. Some of the earlier results may be dismissed, perhaps, on the ground of imperfect technic or unfavorable material, but the same means of elimination can not be used in removing difficulties in more recent observations.

Bardeleben in 1892, the first to make any definite statement of the number of chromosomes in man, claimed it to be sixteen. The number twenty-four was first recorded by Flemming in 1897, if one may disregard the earlier work of Hansemann who also found twenty-four in some cases, but in others eighteen and forty. Wilcox ('00) reported eighteen, with variations of fifteen and nineteen; but whether this represents the diploid or haploid number, he does not say. In 1906, Duesberg, on the basis of finding twelve chromosomes in the first spermatocyte division, corroborated Flemming's conclusion that twenty-four is the unreduced number. In the same year Moore and Arnold described sixteen gemini in the first spermatocyte metaphases, which would make the diploid number thirty-two.¹

In 1910, Guyer published the observation of twenty-two chromosomes in the human spermatogonia. According to him, in

¹In a study of the cytology of malignant growths in man, Farmer, Moore and Walker (Proc. Roy. Soc., B vol. 77, 1906) note the frequent occurrence in mitoses of 32 chromosomes which they consider the normal somatic complement.

the growth period of the spermatocyte the nucleus contains two chromatin nucleoli of unequal size, and occasionally other small nucleolus-like granules which have no constancy in their presence, size or relationship. When the spindle of the first spermatocyte forms, the chromatin nucleoli appear as accessory chromosomes, together with ten bivalent chromosomes. The two accessories pass undivided to one pole of the spindle considerably in advance of the other chromosomes, with the result that one-half of the daughter cells in this division receive twelve, and the other half, ten univalent chromosomes. Since all the chromosomes divide in the second spermatocyte division, one-half of the total number of spermatids receive twelve, and the other half, ten univalent chromosomes.

Gutherz ('12) working over the same ground, disagrees with Guyer's interpretation of the two chromatin nucleoli appearing in the resting nucleus of the spermatocyte. Gutherz finds at this stage in addition to several (1 to 3) true nucleoli, a basophil nucleolus composed of a pair of chromosomes which at times assumes a quadripartite form. This tetrad-like appearance he takes to indicate a subsequent separation in both maturation divisions, which means an equal (quantitative) distribution of chromosomes to all spermatids. Gutherz does not describe the meiotic divisions, nor does he commit himself on the chromosome number. In his figure 10, of a first spermatocyte metaphase, twelve bodies may be counted, but it is by no means certain that they all represent single chromosomes. He seems inclined to accept the results of Duesberg and of Branca (whose paper is not available to me at the present time), both of whom give twenty-four as the unreduced number.

The unanimity of all recent work in settling on twenty-four, or a number very close to it (Guyer, 22 in the male), as the diploid number, is seriously disturbed by the results of Winiwarther ('12) who reports the finding of forty-seven chromosomes in the spermatogonia. According to him, the metaphase of the first spermatocyte shows twenty-four chromosomes, one of which is a heterochromosome that later passes undivided to one pole of

the spindle during the division of the remaining twenty-three. In the second spermatocyte division figures, twenty-three and twenty-four chromosomes are seen, all of which divide; so that one-half of the spermatids receive twenty-three, and the other half, twenty-four.

The difficulties of the problem of the chromosomes in man have not been lessened in any degree by the methods of attack. Thus the conclusions noted above were for the most part based on findings in the germ cells of the testis; while the other aspects of the problem, namely, that of the chromosomes in somatic mitoses, has been entirely neglected. It would appear that attempts at corroborating such conclusions by examination of somatic cells have proved unsatisfactory, and in most cases the somatic number has been arrived at by determining the number in the spermatogonia, or by multiplying the number observed in the maturation spindles by two.

For the purpose of testing the reliability of this method as a criterion for determining the number of chromosomes in the somatic cells, as well as learning by direct observation something about the mitoses in these cells, I undertook the present study. This was made possible by the acquisition of unusually favorable material in the form of an apparently normal human embryo (white) measuring 9 mm. from crown to rump, which was fixed shortly after expulsion in an acetic-bichromate mixture, and cut in paraffin to sections of 10μ thickness.² The sections were stained on the slide with Delafield hematoxylin and orange G, according to the method described by Morris ('09). This staining procedure proved an excellent one not only for general embryological study, but for cytological as well, the chromosomes in many cases standing out with the sharpness of iron-alum-hematoxylin preparations.

²For this embryo I am indebted to Dr. H. L. Woodward of Cincinnati, who obtained it from a case of abortion.

OBSERVATIONS

Mitoses are abundant in every tissue of the body except the endoderm of the alimentary canal, the epidermis and the germ cells, most of which are in the resting condition. The germinative layer of the central nervous system shows the greatest number of division figures. The sex of course could not be determined.

Prophases were found most favorable for study, and at this stage counts could be made with relative ease and accuracy, although in all cases the chromosomes lie at different levels. A scattering of the chromosomes throughout the cell is characteristic and sometimes a partial division with incomplete separation of the halves occurs before the metaphase.

In selecting cells for illustration, I have considered only cells that are uncut by the knife and entirely included in the section. However, conclusions are based on the study of a much larger number, many of which might have been drawn. In many instances it is impossible to determine the number exactly owing to overlapping and crowding; but these are nevertheless useful when interpreted according to other cells indisputably clear. The number of the latter is not great, in spite of the large number of dividing cells in the material; for but relatively few combine the advantages of position in the section and sharp demarcation of the chromosomes from one another.

Figure 1 is from a liver cell. The chromosomes, thirty-four in number, are in the form of rods, usually straight, but sometimes curved, or bent into V's. No attempt has been made to pick out synaptic mates, but it can be readily seen that some of them fall into series of pairs, according to size and form (1, 2, 3).

Figure 2 represents a remarkably clear prophase in a cell from the germinative layer of the brain in which the number of chromosomes is thirty-three. The chromosomes are somewhat thicker than in the preceding cell. Figure 5 is also taken from the brain. This cell is ruptured so that the chromosomes are spread out as in a smear preparation, making the task of counting them a very simple matter. The number is thirty-three or thirty-four, depending upon whether or not we exclude the body marked *z*, which is

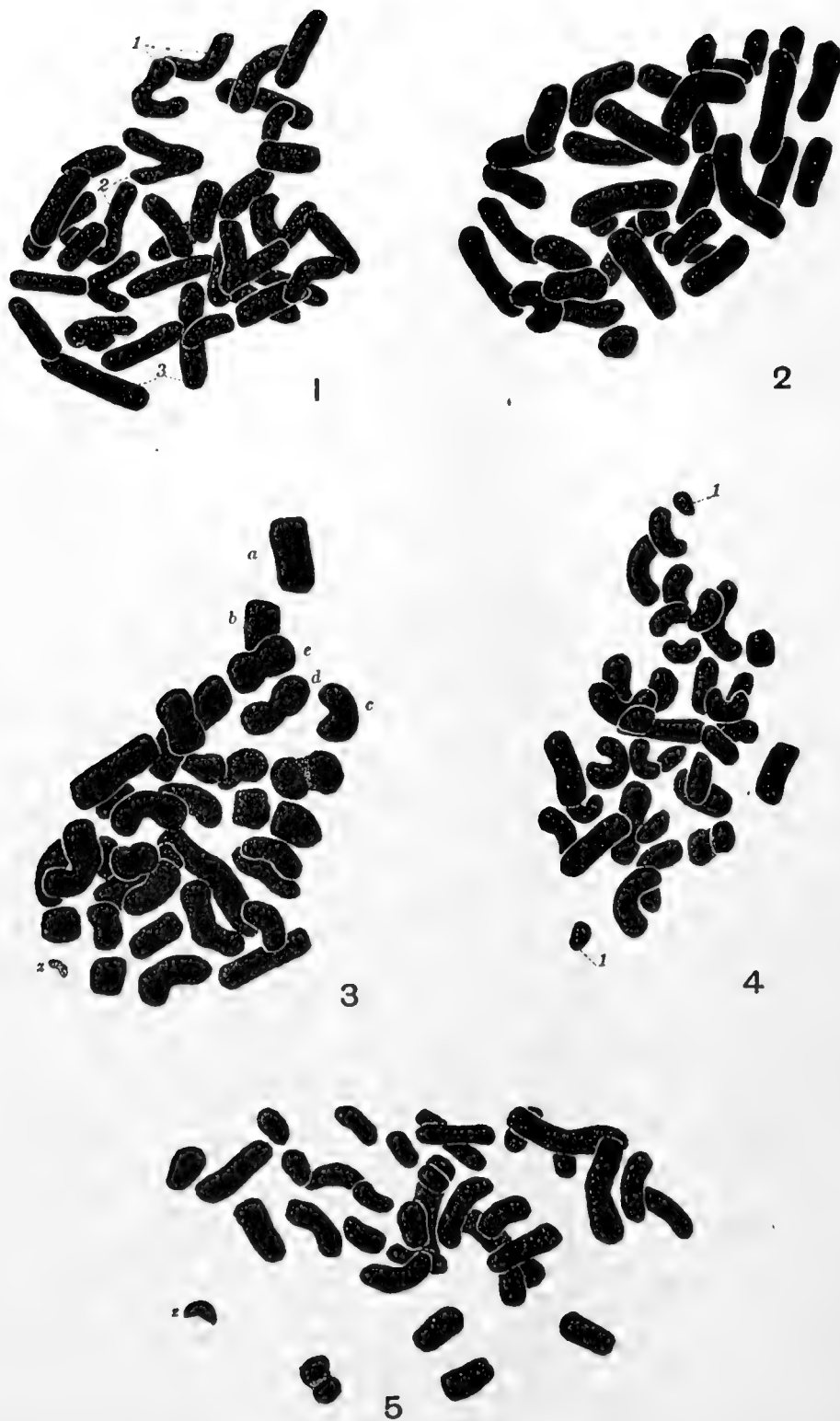
distinguished by its peculiar form and different level, from the other chromosomes. It is perhaps unnecessary to remark that all due precautions were taken in this case as well as others to avoid confusing the chromosomes lying in adjacent cells but different sections.

Figure 3 is from a mesenchyme cell lying directly beneath the epidermis of the ventral body wall, in which the form of the chromosomes differs somewhat from the preceding. At *a* and *b* are two thick heavy bars, and at *e* and *d* bodies resembling the bivalent chromosomes of the maturation spindles. The number is thirty-four, plus a small body *z* which may possibly be a plasmosome fragment.

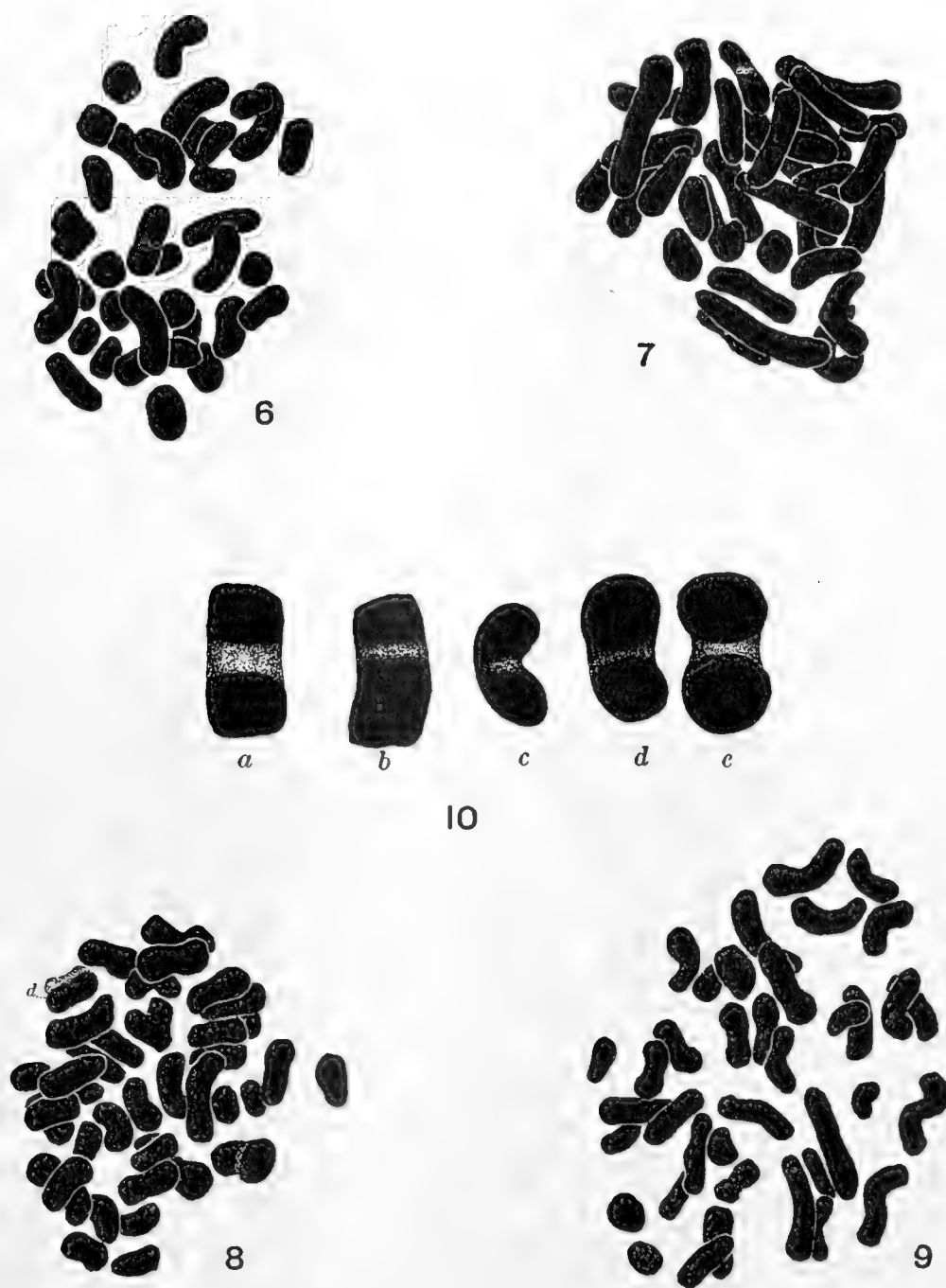
Figure 4, a prophase of a cell in the mesothelium of the intestine, clearly shows thirty-four chromosomes, two to which are characterized by their small size and their location at opposite poles of the group (1).

Figure 6 is a prophase figure from the mesenchyme of the lateral body wall in which the number of chromosomes is thirty-four. Figure 7, taken from a cell in the epithelium of the nasal pit, shows thirty-four chromosomes, many of them in the form of large thick rods. Figure 8 is from a neighboring cell in the nasal epithelium of the same section, in which the chromosomes are much smaller in size but larger in number, thirty-eight. It is possible that some of the chromosomes in this section are partially divided as the one at *d*, and that some of these halves were counted as single chromosomes. I have taken pains to avoid such an error, and I do not believe such a mistake was made; for in the case shown in figure 9, a mesenchyme cell from one of the visceral arches, in which the chromosomes are characterized by their small size, the number can be readily determined and is found to be thirty-eight.

In connection with figure 3, attention was called to the bilobed appearance of several of the chromosomes. Della Valla, Gregoire and more recently Agar, have described similar chromosome-forms in somatic mitoses. When such a chromosome becomes split a tetrad-like body is formed as in figure 8, *d*. Häcker and later Schiller have brought about the formation of typical tetrads



The figures were outlined at table level by means of a camera lucida at the magnification obtained by using a 2 mm. apochromatic objective and compensating ocular 18 (Zeiss); while the details were drawn at a lower magnification. Figure 10 was later enlarged two diameters; all the figures were reduced $\frac{1}{5}$ off in reproduction.



- Fig. 1 Liver cell; 34 chromosomes.
 Fig. 2 Brain cell; 33 chromosomes.
 Fig. 3 Mesenchyme cell; 34 chromosomes.
 Fig. 4 Intestinal mesothelium cell; 34 chromosomes.
 Fig. 5 Brain cell (ruptured); 33, or 34 chromosomes if *z* is counted.
 Fig. 6 Mesenchyme cell; 34 chromosomes.
 Fig. 7 Nasal epithelium cell; 34 chromosomes.
 Fig. 8 Nasal epithelium cell; 38 chromosomes.
 Fig. 9 Mesenchyme cell; 38 chromosomes.
 Fig. 10 Enlarged drawing of chromosomes *a, b, c, d* and *e*, of figure 3.

in the diploid number by treating developing copepod eggs with ether; while Nêmec has caused their formation in plant tissue by means of chloral hydrate.

Agar in his study of the chromosomes in larval *Lepidosiren* sums up his conclusions regarding this form of chromosome in these words: "The tendency for chromosomes to become transversely segmented or constricted is a wide-spread characteristic. It becomes especially operative, but not solely, whenever the chromosomes are short in comparison with their length as happens normally in meiosis and exceptionally in somatic tissue" (p. 295).

One may readily find stages illustrating the steps in such a process. Figure 10 is an enlarged drawing of the chromosomes *a, b, c, d* and *e* of figure 3, which show how the segmentation might be brought about; *a* may be taken as the first step, the reduction in length resulting in a concentration of chromatic material at either end with a thinning out of the middle region. This thinning out which gives the appearance of a transverse segmentation does not always occur in the middle, as may be seen from *b*. In *c* the thin, or more lightly staining region is slightly constricted, and in *s* and *e* the constriction is well marked. The constriction does not represent a line of future division, for it can be clearly demonstrated that division takes place at right angles to it, that is, in a plane passing through the long axis of the chromosome. It is when such a segmented chromosome is beginning to divide that the tetrad-form is produced.

DISCUSSION AND CONCLUSIONS

In the foregoing I have described somatic mitoses in which thirty-three, thirty-four and thirty-eight chromosomes occur. In addition to these I have observed other cases in which the number is thirty-four. In still other instances it is impossible to state the number with certainty, but careful examination of several scores of mitoses leads me to believe that the number thirty-four approximates the one that occurs most frequently in the cells of the embryo under consideration.

The work of Duesberg, Guyer, Branca and Guthertz indicates that the pre-meiotic number is about twenty-four, and that the first spermatocyte metaphases contain one-half this number. *My studies show clearly that in a human embryo the somatic mitoses display chromosomes in a number so much larger than this approximate pre-meiotic one, that the two numbers can not be the same.* One may not always be able to determine with exactness the number of chromosomes, but when the number observed in a great many cases is ten more than the expected average, the values in the two cases must be different.

It is a fact confirmed by countless observations that the number of chromosomes characteristic of the spermatogonia and the ovogonia, that is, the pre-meiotic number, is a constant one, and that the same is true of the meiotic division figures. On the other hand it is also known that the somatic mitoses do not always show a number identical with the pre-meiotic one. That this distinction is not generally recognized is evidenced by the frequency with which 'somatic' and 'spermatogonial' are used interchangeably, although in the classic *Ascaris* embryo the somatic cells undergo chromatin 'diminution' at the very beginning of cleavage. In *Ascaris* the chromosomes in the somatic cells are larger in number though smaller in size than in the germ cells. Krimmel ('10) finds the opposite condition in regard to number in the embryonic and somatic cells of the copepod, *Diaptomus*, in which the chromosome number varies between the reduced and the diploid value.

While it appears that the somatic number in man, though not a constant one perhaps, is different from the spermatogonial number, one should not overlook the results of Moore and Arnold who observed sixteen bivalent chromosomes on the first spermatocyte spindle. The diploid number, thirty-two, would be one closely approaching the number found by me in the somatic cells. Their single figure of three first spermatocytes in division does not show sixteen bivalents in any case, nor does it support their claim in a very convincing manner.

The results of Winiwarther are so at variance with all others that with the evidence at hand it is impossible to interpret them properly. It may be significant that the spermatogonial number

found by him, forty-seven, is about double that found by all recent observers, for this prompts the suggestion that in this case a doubling of chromosomes took place in early development. However, for the present this case must stand as an anomaly, and in view of the conclusions of all other workers in this field can not be accepted as representing a typical condition.

The differences in the form and size of the chromosomes, as shown in the drawings, at first suggested a correlation between these characters and the tissues in which they were observed. Evidence for such an idea can be found in many cells, but is very much weakened by the fact that these distinguishing features are not constant, and the chromosomes of any tissue may appear differently under different conditions.

Figures 8 and 9 show prophases containing thirty-eight chromosomes, a number considerably above the average, thirty-four. Figures 7 and 8 are both from the epithelium of the nasal pit, the former showing thirty-four large chromosomes and the latter thirty-eight, many of which are much smaller. In figure 9 a number of the chromosomes are small in size. The same thing is true of a few other cases where I have observed a relatively large number of chromosomes in the somatic mitoses. These facts suggest that the small chromosomes may be derived by a breaking up or 'diminution' of the larger ones. Likewise the difference between the somatic number and the spermatogonial number (as reported by Duesberg, Guyer and others) may have a similar explanation; but in view of the scanty and questionable character of the evidence, such an explanation can be offered only in a very tentative way. In order to throw more light upon this point, which is a highly important one in reaching any final conclusions in assigning a proper value to the chromosomes in the organization of the cell, a comparative study of mitoses in embryos of the same and different ages, together with an examination of the maturation spindles, is necessary. I have made some headway in securing material for this purpose, which will serve as the basis of a future study.

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THE SPERMIOGENESIS OF THE PRIBILOF FUR SEAL (*CALLORHINUS ALASCANUS* J. AND C.)¹

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THIRTY-EIGHT FIGURES

Notwithstanding the considerable number of investigators, who have studied the spermatogenesis of the mammalia in recent years, many questions remain undecided, and the number of species examined is still but small. The domestic animals and the more common wild forms have furnished practically all the material thus far, and any addition to this number would seem very desirable. The following account of the spermiogenesis in the fur seal, *Callorhinus alascanus* Jordan and Clark, is offered as such a contribution.

The material, assigned to me by Prof. F. M. MacFarland for this study, was preserved in the Pribilof Islands, Alaska, by Mr. G. A. Clark, Special Assistant in Charge of Fur Seal Investigations during the summer of 1909, to whom I desire to extend my sincere thanks for his kind co-operation. Four different fixatives had been used, namely, saturated corrosive sublimate, Bouin's picro-formalin-acetic mixture, Lenhossék's sublimate-alcohol-acetic, and strong Flemming's solution. Small pieces of the fresh testes of two fur seals, one a young male of three years, the other a fully grown adult of eight years of age, were fixed in each of these reagents. All stages in the development of the spermatozoa were found in material from each individual and no important differences were noted, so that this paper is based upon the study of preparations made from both. The sublimate material was dehydrated and iodinated in the usual way and,

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after paraffin imbedding, sections $5\ \mu$ were cut. This was as thin as was found practical, and in most cases was found sufficient for all purposes. After mounting on the slide the sections were treated with a $2\frac{1}{2}$ per cent solution of sodium thiosulphate, diluted with ten volumes of water, to remove all traces of iodine, as recommended by Heidenhain ('09). As for stains, though many others were used, Heidenhain's iron hematoxylin was found by far the most satisfactory, all the elements of the cells being shown in a most favorable manner. The ordinary treatment was used with a slightly longer period of mordantage, the sections remaining in the iron bath for twelve hours at least, and then from twelve to twenty-four hours in the stain. For contrast stains erythrosin, eosin, congo red and orange G were mainly used, the first named giving the best results as it was less likely to overstain and thus obscure delicate structures. This I found to be a serious danger as a heavy stain is likely to cause delicate filaments, such as are seen in the origin of the 'Schwanzmanschette' or caudal tube, or the centrosomes even, to be overlooked. For the study of the mature spermatozoa cover glass smear preparations were made from the epididymis, which has been preserved as a whole in 3 per cent formaldehyde. Further preparations were also made from new material secured by Mr. Clark in the summer of 1912, and the whole development was verified with these.

THE DIVISIONS OF SPERMOGENESIS

In describing the process of the development of the adult sperm from the spermatid I shall follow the plan first laid down by Meves ('99) who separated the process into four main periods. The first extends from the close of the division of the spermatocytes of the II^d order to form the spermatids, up to the appearance of the 'Schwanzmanschette,' or caudal tube; the second extends from this appearance to its final disappearance; the third period from this point up to the migration of the adult sperm into the lumen of the tubule; and the fourth period, that of the so-called 'maturation,' consisting of minor changes in form and density mainly, which take place in the epididymis.

The first period: from the second division of the spermatocytes up to the appearance of the caudal tube: figures 1 to 15

In describing the changes which the spermatid undergoes, there are at least four principal structures to be noticed, the cytoplasm, the nucleus, the idiosome, and the centrosomes. Other important structures appear later and will be mentioned as they occur.

Immediately after the second division of the spermatocytes, the small spermatids are found along the inner surface of the epithelial lining of the tubule (fig. 1). As a rule each is polyhedral, due to the pressure of the adjacent cells. Those spermatids lying nearest the lumen of the tubule appear less influenced by this factor and are often quite rounded. The cytoplasm is clear and transparent, or but slightly granular, and is bounded by a sharply defined cell membrane. The spherical nucleus is at first central in position, but as development proceeds, the whole spermatid becomes distally elongated toward the lumen of the tubule, and the nucleus shifts in position toward the proximal end of the cell. It is comparatively large, resembling both in size and form that of the Von Ebner cells which precede them. So marked is this resemblance that it is often difficult to distinguish between these two stages. The chromatin is scattered, as a rule, throughout the whole area of the nucleus in the form of large irregular clumps which are connected by slender bands of colorless linin (figs. 1 to 5). The nuclear membrane is quite clear and distinct and is generally incrustated on its inner surface with a layer of chromatin of varying thickness. In the closing portion of this period the chromatin begins to change toward the appearance which it has in the adult sperm. The large irregular clumps become resolved into finer granules which are more evenly distributed over the linin network, the nucleus as a whole losing its mottled appearance and taking on a more homogeneous aspect (figs. 11 to 14).

As the end of the period approaches the nucleus also elongates toward the wall of the tubule and shifts bodily in that direction, finally coming to lie at the extreme proximal end of the cell

body (figs. 13 to 15) toward the tubule wall. The prolongation, described by Duesberg ('08) in the spermatid of the rat, which the nucleus sends out to meet the centrosomes is not visible in the fur seal. In the second period the nucleus is often elongated in such a manner, but it is long after the centrosomes have reached the nuclear membrane.

The idiosome, or sphere, of the fur seal shows in most respects those characteristics which have been described by previous authors in mammalian spermiogenesis. It appears shortly after the second division of the spermatocytes as a homogeneous body of considerable size, lying close to the nuclear wall, and usually near its proximal end. It takes an eosin stain a little more deeply than the surrounding cytoplasm, and shows no traces of the cortical granulations or other structural differentiations described for some forms. In shape it is not strictly a sphere but has more the form of a prolate ellipsoid (fig. 1, *s*). The testis of the fur seal is not as favorable material for the study of the development of the acrosome as that of some other forms, but the course of events can be readily followed, and differs but slightly from that described by Benda ('91), Moore ('94), Niessing ('96), Lenhossék ('98), Meves ('99) and others. The first change in the homogeneous idiosome that I have been able to find is the relatively sudden appearance of two densely staining granules, lying in a clearer area within it (fig. 2). The whole sphere stains very faintly at this stage and appears hyaline and semi-transparent. These two granules apparently fuse into a single dense body in the center of the hyaline area (fig. 3). Not all of the sphere is made up of this hyaline substance, however, a denser and more opaque portion lying at one side (fig. 4, *s.r.*) and gradually separating entirely from the clear portion, destined to form the 'capuchon,' or head cap of the mature spermatozoon. This darker remnant of the sphere migrates to the distal part of the cell (figs. 13 to 15) where it finally degenerates along with the cytoplasm of that region, and is cast off in the closing stages. The hyaline body is at first spherical (figs. 3 and 4) but soon begins to flatten against the nuclear wall, its contained central granule, the acrosome, coming in contact with the latter and

likewise flattening against it. This fusion becomes so intimate that it is often impossible to distinguish the acrosome in the later stages of the spermatid. To what the striking variations in size of the acrosome, such as are shown in figures 5 to 9, may be due I am unable to decide.

In the early stages of the contact of the hyaline body with the nucleus the wall of the latter frequently shows a marked degree of flattening, as though yielding to an external pressure, and in many cases the line of contact becomes actually concave (figs. 5 to 7). This condition is, however, but transitory and the convex outline is soon resumed. A marked change also occurs in the substance of the hyaline body now extending back over the nuclear wall. Its substance becomes more dense anteriorly, and stains more readily with eosin. This process continues backward toward the nuclear membrane until the whole head cap becomes transformed into this denser substance. Figure 9 shows a midway stage in which the more anterior part of the head cap has become dense, while the part next to the nuclear membrane is still hyaline. Figures 11 to 15 show the head cap entirely composed of the denser substance. During this differentiation the process of flattening and overgrowth is continuing, so that by the end of the first period the head cap has extended well down over the anterior half of the nucleus. It is manifest from the above that the head cap reaches nearly its adult form in the first period of spermiogenesis, and its subsequent development consists mainly in a process of further differentiation, no new elements being added to the form which it has already assumed.

The first appearance of the centrosomes in the young spermatid is at the periphery of the cell, in the form of two small, rounded granules, one a little larger than the other, lying close to the cell membrane (fig. 1, *c.*). Actual proof of the centrosomal nature of these granules in the fur seal is lacking, as I have not traced their earlier history. From subsequent events, however, and from analogy with other forms in which their history is fully known, I deem it safe to consider them as the centrosomes. In the earliest stage in which I have been able to detect them there

is no trace of a tail filament. It soon appears, however (fig. 10, *a.f.*) and may be readily found in cases in which the centrosomes have migrated inward to their contact with the nuclear membrane, as in figures 11 and 12. In nearly every instance the tail filament projects out freely beyond the boundary of the cell, and even in such an early stage as that shown in figure 10, it has already reached a considerable length. The appearance and growth of the tail filament must be extremely rapid, for it is very difficult to find any early stages. The migration of the centrosomes toward the nucleus must be quite rapid also, as intermediate stages between those shown in figures 10 and 11 are very rare. No indication of any prolongation extending from the nucleus toward the centrosomes, such as described by Meves ('99), and by Duesberg ('08), could be found, and the migration seems to be entirely an active one on the part of the centrosomes, so far as such a visible participation of the nucleus is concerned.

After reaching the nucleus the anterior centrosome becomes closely pressed up against the membrane (figs. 11 and 12) and at the end of the first period it is fused with the membrane, often almost disappearing from view in the chromatin incrustated wall. In this fusion the anterior centrosome is lengthened in a direction at right angles to the tail filament. In many instances it appears as if the centrosome had penetrated the membrane and was situated on its inner surface as in figures 13 and 14.

The second period: from the appearance of the caudal tube to the migration of the distal half of the posterior centrosome (the annulus) along the tail filament: figures 16 to 29 .

In the original division of Meves ('99) the second period extends from the origin of the 'Manschette' up to its total disappearance from the cell. As will be shown in the following pages the 'Manschette' does not disappear in the developing spermatid of the fur seal, but persists and takes an important place in the structure of the adult sperm. For this reason we are compelled to seek another phenomenon which, if possible, occurs constantly at the time of the disappearance of the caudal tube in other

forms, that we may limit the present period in our material. Meves ('99) in his work on the guinea-pig states that the disappearance of the 'Manschette' is synchronous with the beginning of the movement of the annulus along the tail filament, a statement later confirmed for the rat by Duesberg ('08). The general parallelism of events in the fur seal spermiogenesis with that in other mammals leads us to consider this migration of the annulus down the tail filament as marking the close of the second period.

Retzius ('09, p. 227), has well pointed out that the term 'Manschette' is not a well selected name for a structure around a neck, and that 'Halskragen' is not much better, since both imply an opening at one side, either open or buttoned. As the structure in question is a true tube, he prefers the older name 'Schwanzröhre,' which I have adopted in this description in the translated form 'caudal tube.'

The cytoplasmic body of the spermatid at the beginning of this period is still more or less polygonal in section, with the nucleus more or less shifted proximally from its central position. During this stage the whole cell becomes elongated in a direction radial to the tubule, and the nucleus approaches the proximal end of the cell until it reaches the surface, the cytoplasm being massed toward the lumen while the cell membrane and acrosome cover the apical portion of the nucleus. At the beginning of this period the manschette or caudal tube appears, one of the most striking phenomena observed in mammalian spermiogenesis. The riddle of its origin and fate has interested some of the most prominent workers in cytology. A brief review of the literature will be given at the close of the present paper, while the description of personal observations will be alone presented here.

Shortly after the centrosomes and their tail filament have reached the nuclear membrane there appears in the cytoplasm surrounding the axial thread a series of delicate filaments attached to the nuclear membrane. The proximal ends of these arise in a circle around the basal end of the nucleus with the centrosomes as a center, while their distal ends project freely into the cytoplasm in directions at various angles to the axial thread which they surround (figs. 16 to 19). The first form of the caudal

tube is that of a 'Faserkorb,' or basket work, similar in form and origin to that described by Meves ('99) for the guinea-pig. These fibrils are at first very short and thin, but they increase in length and thickness rapidly. By the progressive differentiation of the cytoplasm between them they soon fuse into a hyaline tube, surrounding the axial thread and open at its lower extremity. In optical section the sides of this tube appear as two strong curved lines as shown in figures 20 to 28. The line of attachment to the nuclear wall is soon marked by a thickening of the edge of the caudal tube, which appears as a rounded knob when seen in optical section (fig. 27). The cytoplasm included within the caudal tube stains more readily also than that portion outside of it. Progressively the amount of cytoplasm surrounding the caudal tube becomes reduced more and more as the main mass continues its migration toward the lumen of the tubule, soon leaving but little more than the cell membrane investing the nucleus and the caudal tube.

The nucleus of the spermatid undergoes great changes in this period, which affect both its form and its staining character. The fine granules, into which the irregular clumps of chromatin of the early stages have resolved themselves become still smaller and more uniformly distributed, and give rise to the homogenous appearance characteristic of the adult sperm. The staining character of the nucleus shows marked changes in respect to iron hematoxylin. It now stains very deeply, and loses scarcely any color on differentiation, so that the head has a deep brown or black appearance. In the later stages much of this staining affinity is again lost, so that it appears a light bluish gray. The shape of the nucleus is also steadily changing during this period, a decided flattening becoming more and more evident as development proceeds, differentiating a dorsal and a ventral surface from the narrowing sides.

The head cap has been practically completed in the former period, and its further development consists in a flattening and condensation of its earlier form, thus making a more intimate union with the nucleus, extending down over the whole anterior and middle portion of the latter, and thinning away behind to

a line. In this way the perforatorium is formed, a structure less conspicuous in the fur seal than in many other mammalia.

In the earlier part of this period the remnant of the sphere is visible in the distal region of the cell as an irregular mass, slightly denser than the surrounding cytoplasm. Here it undergoes a gradual degeneration, eventually disappearing from view.

Shortly after the fusion of the anterior centrosome with the nuclear wall, which takes place near the end of the first period, the posterior centrosome undergoes a division into two equal parts (figs. 21 to 23). At about the same time there appears a rod-like prolongation from the anterior centrosome, extending obliquely downward and outward in the cytoplasm inclosed by the caudal tube. This is the so-called 'batonnet,' or rodlet (figs. 20 to 25), a well marked structure in the fur seal. It increases in length steadily and finally touches the wall of the caudal tube (fig. 27).

Following the division of the posterior centrosome its distal half migrates along the axial filament to take up its definitive position at the end of the connecting piece. As this marks the beginning of the third period it will be described in that connection.

The vesicle described by Meves ('99) for the guinea-pig, and by Duesberg ('08) for the rat, upon the tail filament, is also present in the fur seal, but has been observed in only a few cases. It appears to be of similar origin and relation to the tail filament, but its history has not been followed in this material.

The third period: from the migration of the annulus along the tail filament to the passage of the spermatozooids into the lumen of the tubule: figures 30 to 32 and 34 to 35

The progressive lengthening of the spermatozoid has now shifted the main mass of the cytoplasm to the distal end, leaving only a thin layer covering the caudal tube, the cell membrane appearing as a delicate line. In many preparations this is seen with difficulty, especially as the spermatids soon enter into rela-

tion with the cells of Sertoli, burying their heads in a broad prolongation of the cytoplasm of these cells, and thus becoming closely packed together. The cell membrane can be traced forward over the nucleus in many cases, however, and undoubtedly persists in the adult sperm, forming the semi-permeable limiting membrane described by Koltzoff ('09) as occurring in the spermatozoa of various species of animals. Over the surface of the future connecting piece this cell membrane is not to be confounded with the much more strongly differentiated walls of the caudal tube, which stand out in optical section as two curved lines approaching each other distally, and sharply set off from the surrounding cytoplasm. Within the tube the cytoplasm tends to become denser and stains darker than before. At the proximal end the line of attachment of the tube to the nuclear membrane becomes marked by a circular ring-like thickening which stands out clearly, and in optical section as shown in figure 27, presents the appearance of a spherical knob at the junction of tube wall and nucleus. In cross section (fig. 26) the thickened wall of the caudal tube presents an elliptical outline, near the center of which may be seen the centrosome or the caudal filament, depending upon the location of the section. At about the beginning of the migration of the annulus a marked change is observed in the caudal tube. It becomes detached from its original line of union around the distal end of the nucleus, and undergoes a continuous shrinkage, so that it finally lies below and apparently free from the nucleus (figs. 29 to 32). Another result of this shrinkage is a lateral displacement of the caudal tube, apparently due to the projection of the rodlet against the wall of this structure. When the shrinkage occurs this wall is apparently prevented from approaching the tail filament, while the opposite wall, having no such resistance, approaches the tail filament freely until it nearly reaches it (fig. 32). Shortly after this process of shrinkage is completed the rodlet suddenly disappears. What its fate may be is not clear, but it is clearly evident that it takes no part in the formation of the spiral filament surrounding the tail thread, as described by Schoenfeld ('00) for the bull, and by Van Mollé ('06) for the squirrel.

After the disappearance of the rodlet still further shrinkage takes place, especially at the more posterior part of the connecting piece, with the result that the wall of the caudal tube closely parallels the caudal filament but a short distance from it, save at the anteriorend where a marked swelling indicates the location of the proximal group of the posterior centrosomes (figs. 34 and 35). This enlargement gradually becomes eliminated in later stages and the connecting piece finally assumes its uniform cylindrical shape in the epididymis during the fourth period. The cell membrane covering the caudal tube, has fused indistinguishably with the latter, which terminates anteriorly at the beginning of the connecting piece. The membrane, however, is continued forward over the neck region and is lost to sight in its intimate fusion with the nuclear wall and with the perforatorium in front. Within the connecting piece the cytoplasm increases in density and appears more granular, while the tail filament also becomes thicker and more strongly defined. These granules stain with the Benda method and are apparently of a mitochondrial nature, but the fixation of the material did not permit the tracing of their complete history.

The nucleus has progressively condensed and flattened. Its staining capacity with respect to iron hematoxylin has changed from that of the previous period. The deep black color is rapidly differentiated and the whole nucleus becomes a light bluish gray, the darker transverse bands or zones of Valentin, described in detail by Ballowitz ('91) for other forms, becoming clearly visible in most instances.

When last considered in the previous period, the centrosomes consisted of two parts; first, an anterior centrosome fused with the nuclear wall, from which the rodlet projected out obliquely into the cytoplasm surrounded by the caudal tube, and, second, the posterior centrosome, which had also divided into two parts, a proximal and a distal. The distal half soon starts on its migration down the tail filament. The distinct ring form cannot be made out since it fits closely to the axial filament, upon which it appears more as a bead upon a thread than as a loosely fitting annulus. The migration is quite rapid apparently, as but

few stages could be found in which the annulus was in the course of its movement.

After the annulus has reached its final position, about $6.0\ \mu$ from the nucleus, a striking change takes place in the proximal half of the posterior centrosome. It becomes lengthened in a direction at right angles to the axial filament, at the same time becoming slightly bent, so that it consists of two arms, one continuous with the tail filament, and the other at right angles to it (fig. 30). The point of junction of these two arms now thins away, and finally breaks through, leaving the centrosome divided into two usually unequal bodies, lying side by side in the proximal end of the caudal tube (figs. 31 and 32). This division occurs at about the same time that the staining quality of the nucleus begins to diminish, a change which permits the ready recognition of the anterior centrosome again (fig. 30). It has also elongated in a direction transversal to the axial filament, across the base of the nucleus, and has also divided into halves. These two bodies become later attached by thin filaments, each to its corresponding fellow of the posterior centrosome (figs. 34 and 35). These filaments are imbedded in a homogeneous hyaline substance, and enclosed externally by the delicate cell membrane, all together forming the neck of the adult spermatozoon. The structural weakness at this point accounts for the frequency with which the head is seen broken away from the connecting piece.

At the completion of the changes outlined in the foregoing the spermatozoon has nearly attained its adult form, save for an irregularly rounded mass of cytoplasm, which is still attached to the connecting piece. The most of the cytoplasm of the spermatid, however, has broken up into rounded masses which have become detached and lie along the boundary of the lumen of the tubule. The spermatozoon itself now becomes free in the lumen and passes over into the rete testis, and from thence to the epididymis, where the last remnant of the adherent cytoplasm is lost. In this region what may be considered as the mature spermatozoa are to be found.

The mature spermatozoon

Most of the following points were made out by the study of smear preparations of the contents of the epididymis from material which had been preserved in 3 per cent formaldehyde. The preparations were stained in a large variety of ways, and studied in water, in dilute glycerine and in balsam.

The only descriptions of the spermatozoa of the Pinnipedia thus far to be found in the literature are those given by Ballowitz ('08) for *Phoca vitulina* L., and by Retzius ('09 a) for *Halichaerus grypus* Fabr. In general form the spermatozoon of *Callorhinus* is similar to those though differing in dimensions and in details. The following table gives a comparison of the dimensions of

TABLE 1

MATURE SPERM OF	TOTAL LENGTH	HEAD		NECK LENGTH	TAIL			TOTAL LENGTH TAIL
		Length	Width		Con- necting piece	Main piece	End piece	
	μ	μ	μ	μ	μ	μ	μ	μ
<i>Phoca vitulina</i> L..	68.0	6.0	4.0		12.0			58.0
<i>Halichaerus gry-</i> <i>pus</i> Fabr.....	33.7	8.0	5.5		8.0			25.7
<i>Callorhinus alas-</i> <i>canus</i> J. and C..	54.12	5.95	3.74	0.56	6.1	39.1	2.41	47.6

the three forms. The figures for *Phoca* are taken from the description of Ballowitz, while those of *Halichaerus* must be regarded as approximate only, having been computed from the plates of Retzius, he giving no exact measurements in figures of this species.

Caput. The head of the spermatozoon of *Callorhinus alascanus* J. and C. (fig. 33) is of the form common among carnivora, being oval in outline, the anterior margin rounded, the posterior one abruptly truncate and somewhat emarginate in the center. The whole structure is flattened dorso-ventrally, one of its faces being slightly more so than the other. In profile view it is lanceolate, with an acuminate point (fig. 36). The head is covered by the galea capitis, or head cap (fig. 33, *h.c.*,) the anterior margin of which forms the cutting edge of the perforatorium. The head

cap attains a maximum thickness of $0.8\ \mu$ in front and gradually thins away to its posterior edge, which is more or less clearly visible in face view as a slightly curved line or band. Behind this follows a lighter zone, ca., $1.5\ \mu$ in width, succeeded in turn by a darker band, which extends to the posterior end of the head.

Collum. The neck (fig. 33, *n.*) includes in front the anterior centrosomes, and extends posteriorly as far as the anterior portions of the posterior centrosome, but does not include them. The noduli anteriores or anterior centrosomes are two in number and are closely fused to the posterior wall of the head, and, lying in slight depressions, often appear to be included within the nucleus itself. In figure 38 a case is represented in which the head had been detached from the neck, leaving the anterior centrosomes with the latter. In a few instances there seemed to be present a third anterior centrosome between the other two, but in the great majority of cases two only could be made out. The remaining portion of the neck is made up of the massa intermedia, a homogeneous substance with no discernible structure. Through this substance stretch two delicate filaments uniting the anterior centrosomes to the succeeding ones, while around the whole neck a delicate membrane, a part of the original membrane of the spermatid, forms the outer boundary. The attachment of the neck to the head is usually asymmetrical, it being displaced to one side of the median line (fig. 33). In other cases, fewer in number, it may be exactly centered, or practically so (fig. 37).

Cauda. The tail is divided into three parts, the connecting piece, the principal piece, and the terminal piece.

Pars conjunctionis. The connecting piece (fig. 33, *c.p.*) includes the proximal half of the posterior centrosome, and is limited posteriorly by the distal half of the same, the annulus, or end disc. The noduli posteriores are two in number, approximately equal in size in the adult sperm, though a decided difference in the earlier stages is the rule. From one of these the filum principale, or axial filament extends throughout the whole length of the tail. Surrounding this filament is a very compact sheath of

cytoplasm, in which numerous granules may be distinguished, but containing no regular spiral fiber. Outside of this and forming the external sheath of the connecting piece is the involucre externum, which, as we have seen, is made up of the wall of the caudal tube plus the cell membrane of the spermatid covering this portion. The connecting piece is terminated by the annulus, or distal half of the posterior centrosome. In the adult sperm it stains but slightly and is very inconspicuous, but in favorable cases may be made out as a narrow transverse disc, pierced in the center by the axial filament. In figures 33 and 36 there still remains a small amount of cytoplasm, *c.r.*, attached to the connecting piece, but this soon disappears. In the same preparation in which the spermatozoon represented in figure 33 was found there were many others in which the outline of the connecting piece was perfectly cylindrical, this particular one having been selected for other reasons.

Pars principalis. The main, or principal piece of the tail (fig. 33, *m.p.*) consists of the tapering axial filament, covered by a homogeneous sheath, and extends from the annulus to the point where the naked axial filament continues alone beyond the sheath surrounding it. The diameter of the principal piece gradually decreases throughout its whole extent, so that it is difficult to determine the exact point at which the sheath stops and the final segment of the tail begins in every instance.

Pars terminalis. The end piece (fig. 33, *e.p.*) of the fur seal spermatozoon is short, measuring approximately $2.5\ \mu$ in length. It appears to be made up of the single axial filament alone. In many instances it appeared to be broken, and the limitations of the material at hand prevented any satisfactory study of this extremely delicate object.

GENERAL DISCUSSION

No attempt will be made here to review the voluminous literature upon spermiogenesis, since so many masterly summaries, such as those of Meves ('99 '01), Waldeyer ('06) and Duesberg ('08), already exist and make it entirely unnecessary. The relation of certain points as brought out in the fur seal spermiogenesis should be here emphasized, however.

The caudal tube was held by the earlier writers, such as Koelliker ('67), to be derived from the nuclear membrane, a view upheld today in a modified form by Schoenfeld ('00) for the bull, and by Van Mollé ('06 '10) for the squirrel, the mole, the guinea-pig and the mouse. According to the last cited author a hernia-like circular fold of the nuclear membrane is formed, which grows backward as a double-walled tube. Between the lamellae of this tube is a clear fluid derived from the nuclear sap. When the annulus migrates downward along the axial filament it takes with it the inner membrane of this tube, evaginating it completely. At the same time the whole wall contracts so that the final length of the single-walled tube is the same as when in the earlier double-walled condition.

Opposed to this conception is that of the derivation of the caudal tube by a process of cytoplasmic differentiation alone, as held by Lenhossék ('98), Meves ('99), Duesberg ('08 '10), Retzius ('09 b '09 c), Branca ('09), Le Plat ('10) and many others. All of these agree that the caudal tube arises with comparative abruptness, its earliest beginnings being more or less obscure and difficult to follow. Meves has given the most detailed account of the process. According to his observations on the guinea-pig a series of filaments inserted in a circle upon the nuclear membrane at first appear. These are at first oblique, giving an hour-glass outline to the whole structure. Progressively they become more nearly parallel to the main axis of the future spermatozoon and are united by a homogeneous substance into a hyaline tube, the 'Manschette.' The fibrillar stage appears to be very short and has been seen in detail by but few observers besides Meves.

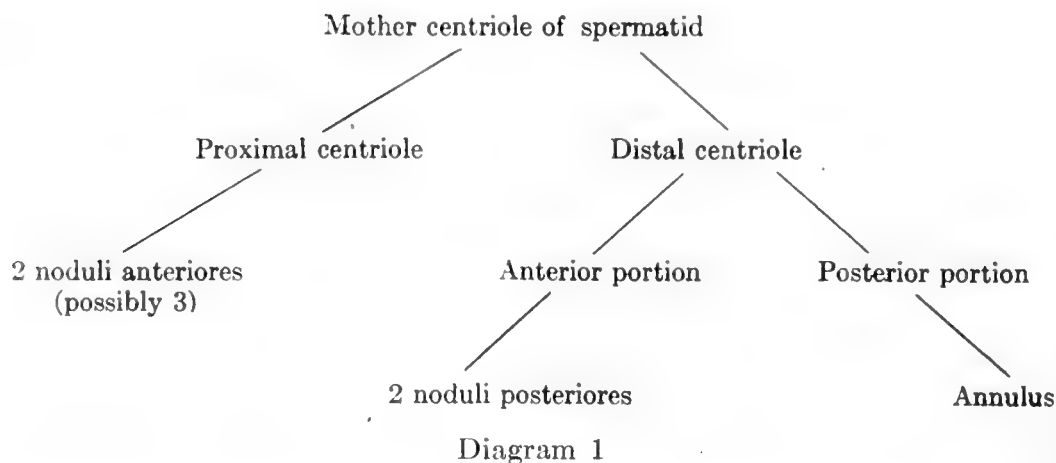
My study of the events in the fur seal gives a complete confirmation of the origin of the caudal tube as held by Meves, the early fibrillar stages shown in figures 16 to 19 being clearly visible though not abundant, in my preparations.

In respect to the ultimate fate of this structure two views are held. According to Koelliker ('67), Meves ('99), Schoenfeld ('00), Duesberg ('08), Branca ('09), and Le Plat ('10) it disappears without leaving any trace, and its function throughout is problematical. Retzius ('09) has emphasized the fact that the caudal

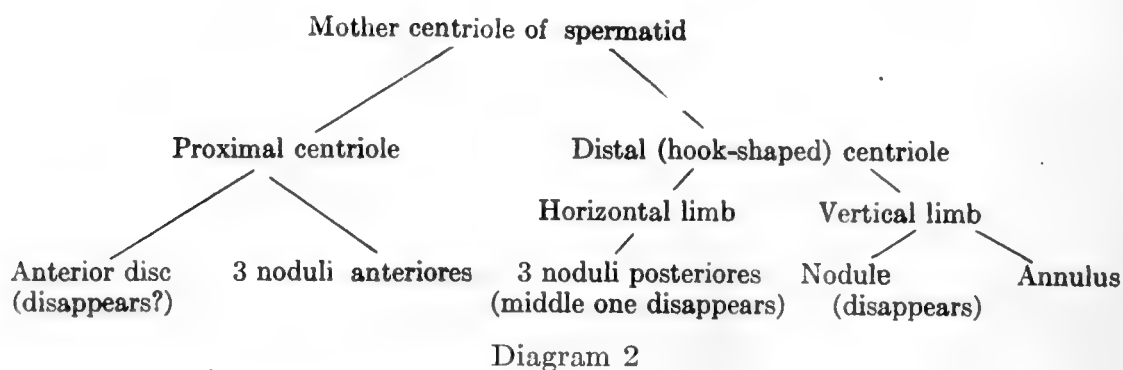
tube is seldom present after the annulus has begun its migration along the axial filament, and suggests that its possible function may be that of a protection to the centrosomal apparatus, shutting out the mitochondria from that region until the differentiation of the annulus and the other centrosomal structures may be completed. No such explanation is adequate for the fur seal, since the caudal tube may be readily followed from its first appearance up to its final incorporation in the connecting piece as a peripheral layer, or sheath. In this instance it forms a permanent structure in the adult spermatozoon, a fate in harmony with the second view, held by Klein ('80) Biondi ('85), Hermann ('89), C. Niessing ('96), Lenhossék ('98), and Van Mollé ('06).

The function of the rodlet which grows out from the proximal centriole is here as elsewhere unsatisfactory. It appears to act mechanically during the shifting and shrinkage of the caudal tube, as shown in figures 29 to 32, but what advantage this may be is not evident, nor is it at all probable that this is its only function. Shortly after this stage it disappears completely. No trace can be observed of its being utilized in the formation of the spiral filament of the connecting piece, contrary to the opinion of Van Mollé ('06).

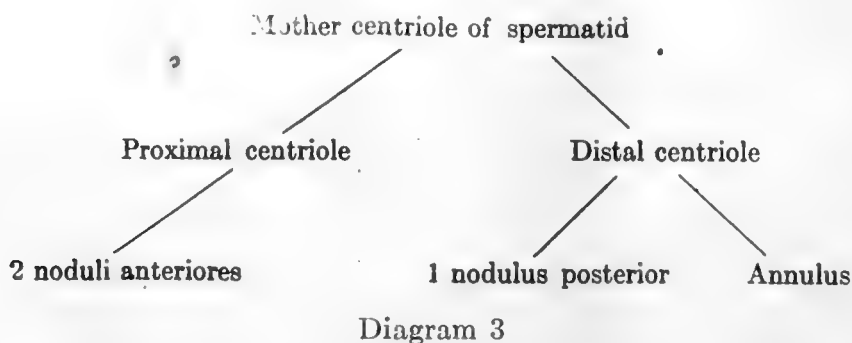
The behavior of the centrioles follows the general plan apparently common to all mammals, being simpler than that described by Meves ('09) for the guinea-pig, and agreeing closely with that seen by Retzius ('09). The proximal centriole of the spermatid divides into two portions, closely adherent to the nuclear wall, each connected by a filament to one of the distal group. The distal centriole divides into an anterior and a posterior portion. The posterior portion becomes the annulus, while the anterior one divides again, forming the *Noduli posteriores*. These changes may be graphically expressed by diagram 1.



A similar diagram, modified from Heidenhain ('07), representing the more complicated phenomena in the guinea-pig is introduced for comparison (diagram 2).



Le Plat ('10) has described a much simpler condition for the cat, which may be expressed by diagram 3.



Le Plat considers that the two small granules at the end of the axial filament are merely thickenings of the anterior ends of the latter and that they are not of centrosomal origin.

From a consideration of the diagrams it is readily seen that the fundamental nature of the transformations of the centrioles is the same throughout, the differences appearing in smaller details, and it is not at all improbable that studies upon other forms may bring out still further complications. No other branch of cytology, however, presents greater difficulties than the study of these minute objects, and great caution must be used in criticism and in generalization from a limited number of facts.

In conclusion, I desire to express my indebtedness to Prof. F. M. MacFarland for the constant interest he has taken in my work, and for the final revision of my manuscript, and to Mrs. Olive H. MacFarland for her great kindness in redrawing the figures which illustrate it.

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ABBREVIATIONS

<i>a.</i> , acrosome	<i>h.c.</i> , head cap
<i>a.f.</i> , axial filament	<i>h.n.</i> , nucleus of spermatozoon
<i>an.</i> , annulus	<i>m.p.</i> , main piece of adult spermatozoon
<i>c.</i> , centrioles	<i>n.</i> , neck of adult spermatozoon
<i>c.p.</i> , connecting piece	<i>n.a.</i> , anterior nodules (centrioles)
<i>c.r.</i> , cytoplasm remnant	<i>n.p.</i> , posterior nodules (centrioles)
<i>c.t.</i> , caudal tube	<i>p.c.</i> , posterior centrioles.
<i>d.c.</i> , distal centriole	<i>r.</i> , rodlet
<i>e.p.</i> , end piece of adult spermatozoon	<i>s.</i> , idiosome, or sphere
<i>f.</i> , caudal tube filaments	<i>S.c.</i> , cytoplasm of Sertoli cell
<i>h.</i> , hyaline portion of idiosome	<i>s.r.</i> , idiosome remnant

All figures are reproduced at a magnification of one thousand diameters.

Figs. 1 to 15 First period of spermiogenesis

Fig. 1 Spermatid shortly after last division of spermatocyte, the idiosome homogeneous, a pair of centrioles (?), *c.*, lying in the cytoplasm below the nucleus.

Fig. 2 Spermatid showing two deeply staining granules in the idiosome.

Fig. 3 Spermatid with idiosome containing a single large central body, the acrosome anlage.

Fig. 4 Spermatid with idiosome separating into the sphere remnant, *s.r.*, and a hyaline portion, *h.*, containing the dense central body, the acrosome.

Fig. 5 The idiosome begins to flatten against the nuclear wall, which is also slightly flattened. The acrosome is in contact with the nuclear wall and has fused with it.

Fig. 6 Continuation of flattening of idiosome and acrosome against nuclear wall. The chromatin of the nucleus is becoming more finely divided.

Fig. 7 The pressure of the idiosome against the nuclear wall has apparently produced an inbending of the latter. In the cytoplasm at the right of the sphere remnant is a large deeply staining body.

Fig. 8 The idiosome is extending backward around the anterior portion of the nucleus in a cap-like form. The acrosome is also much flattened along the nuclear membrane.

Fig. 9 A differentiation has appeared in the substance of the idiosome, or head cap, its outer anterior portion becoming much more dense, a narrow hyaline portion still intervening between this and the nucleus.

Fig. 10 Spermatid with block-like masses of chromatin in the nucleus. The idiosome is not shown in the section. At the lower boundary the centrioles are seen, with the axial filament extending from the distal one out freely into the lumen of the tubule.

Fig. 11 The head cap substance has become equally dense throughout. The acrosome has extended along the surface of the nuclear wall below the head cap, and has fused indistinguishably with it, appearing now as a thickened line co-extensive with the under surface of the head cap. The centrioles have migrated inward toward the nucleus, with which the proximal one is now in contact. From

the distal centriole the axial filament extends through and beyond the cytoplasm of the spermatid. In the distal cytoplasm are a few deeply staining rounded masses. The nucleus is shifting toward the proximal end of the cell.

Fig. 12 Similar to figure 11, but slightly more advanced.

Fig. 13 The proximal centriole has fused with the nuclear membrane and is apparently within the nucleus itself. At the right of the latter the remnant of the sphere is seen.

Fig. 14 A stage similar to the preceding one save that the acrosome is more clearly seen. The nucleus has shifted still further toward the proximal end of the cell, its chromatin is finely divided, and the whole nucleus begins to stain a more uniform dark color.

Fig. 15 The nucleus is at the periphery of the cell, enveloped only by the cell membrane, which is itself indistinguishable. The proximal centriole is not yet in contact with the nuclear wall.

Figs. 16 to 29 Second period of spermiogenesis

Fig. 16 A slightly smaller spermatid than usual, probably due to the plane of the section. The earliest appearance of the caudal tube filaments is shown. The centrioles and axial filament were not included in the plane of the section. Nucleus at the proximal pole of the cell, its chromatin now reduced to fine granules, the whole structure staining dark.

Fig. 17 A similar stage to the foregoing except that the centrioles and axial filament are shown. The latter extends out obliquely between the filaments, crossing two of them.

Fig. 18 Early stage of formation of filaments of caudal tube. The axial filament from the distal centriole passed out from the plane of the section before reaching the cell boundary.

Fig. 19 The general dark tone which the nucleus takes on at this stage is here represented. Many scattered small granules of chromatin are still visible. Four of the filaments to form the caudal tube are visible in this focus.

Fig. 20 The nucleus has become more elliptical and more homogeneous. The filaments have fused together into a hyaline caudal tube, the sides of which are distinguishable as sharply defined lines. From the proximal centriole a delicate rodlet has grown outward and backward. The distal centriole has increased in size. Beyond the opposite pole of the nucleus the thickened head cap appears, the acrosome showing as a heavy line.

Fig. 21 The nucleus presents the form of a double pyramid, its anterior portion lighter and more granular than the posterior part, which is dense and dark. A short rodlet projects from the anterior centriole. The posterior centriole shows a constriction as if dividing. The portion of the nucleus included within the caudal tube often has concave outlines for a short time, as here shown in this stage.

Fig. 22 Differentiation of nuclear areas not clearly shown. The nucleus is covered by a thick head cap with well defined acrosome. The distal centriole has divided into an anterior and a posterior half.

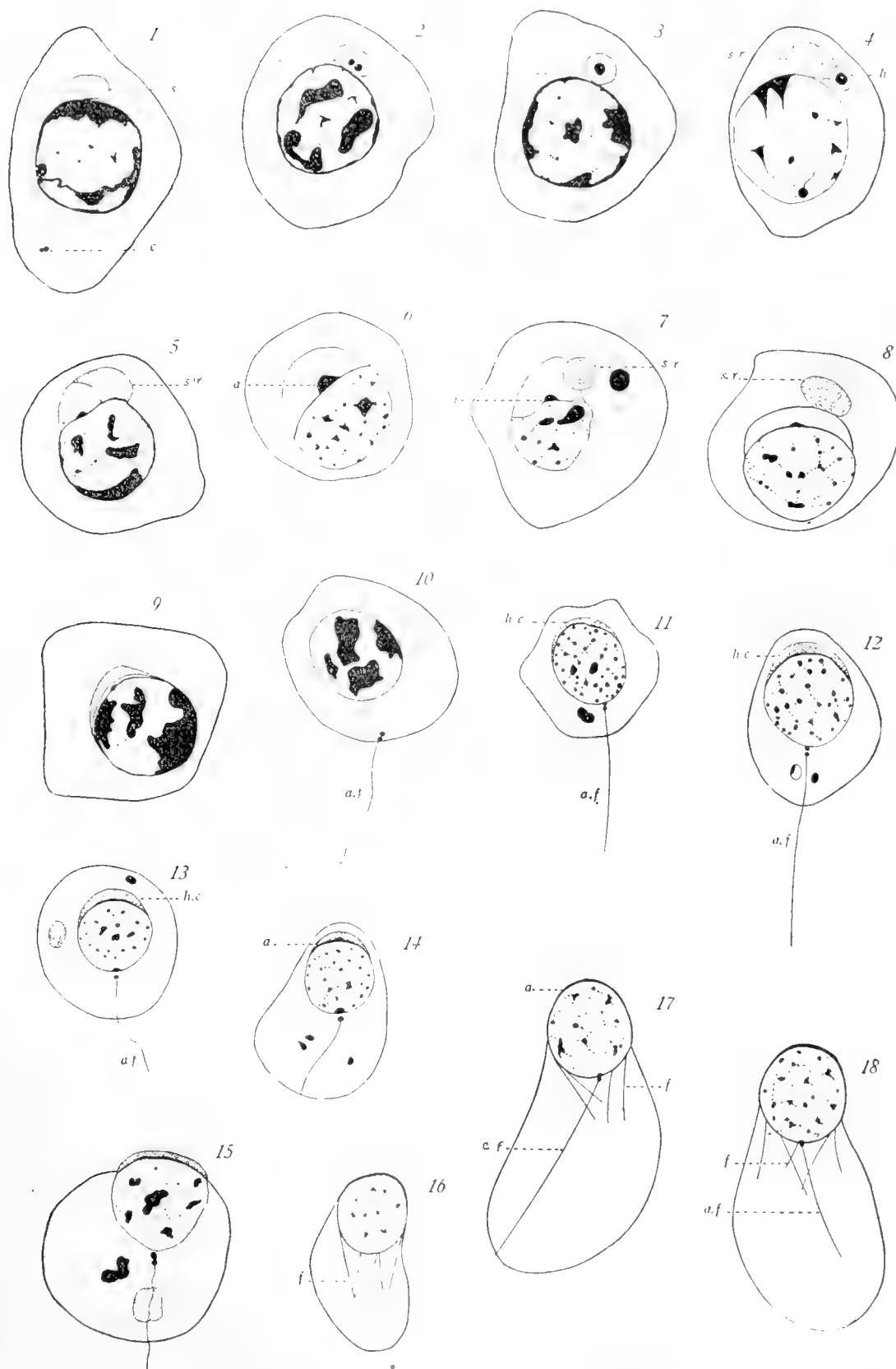


Fig. 23 Oblique view of a slightly flattened spermatid. A large clear cytoplasmic area surrounds the anterior portion of the nucleus, the limits of the head cap not being evident in it. The nucleus is denser, especially its posterior portion.

Fig. 24 Face view of spermatid. The nucleus is uniformly dark, the rodlet has lengthened and nearly reaches the caudal tube. The caudal tube is much longer and its included cytoplasm now stains darker. In the head cap the acrosome is not distinguishable as a separate structure, the two uniting in a heavy line.

Fig. 25 First appearance of transverse bands across the nucleus. The upper dark area coincides with the portion covered by the head cap.

Fig. 26 Cross sections of two spermatids at the level of the centrioles showing the flattened elliptical outline of the caudal tube in this view.

Fig. 27 As the spermatid elongates the cell membrane approaches the wall of the caudal tube in its proximal portion. The proximal centriole is much flattened and scarcely visible. The rodlet has reached the inner surface of the wall of the tube. The insertion of the caudal tube upon the nuclear wall is marked by a circular thickening, knob-like in cross section.

Fig. 28 Profile view of elongated spermatid. The considerably flattened nucleus shows cross-banding. The head cap has stained black at the tip. The remnant of the sphere has shifted to the distal portion of the cytoplasm, and appears as a faint outline only.

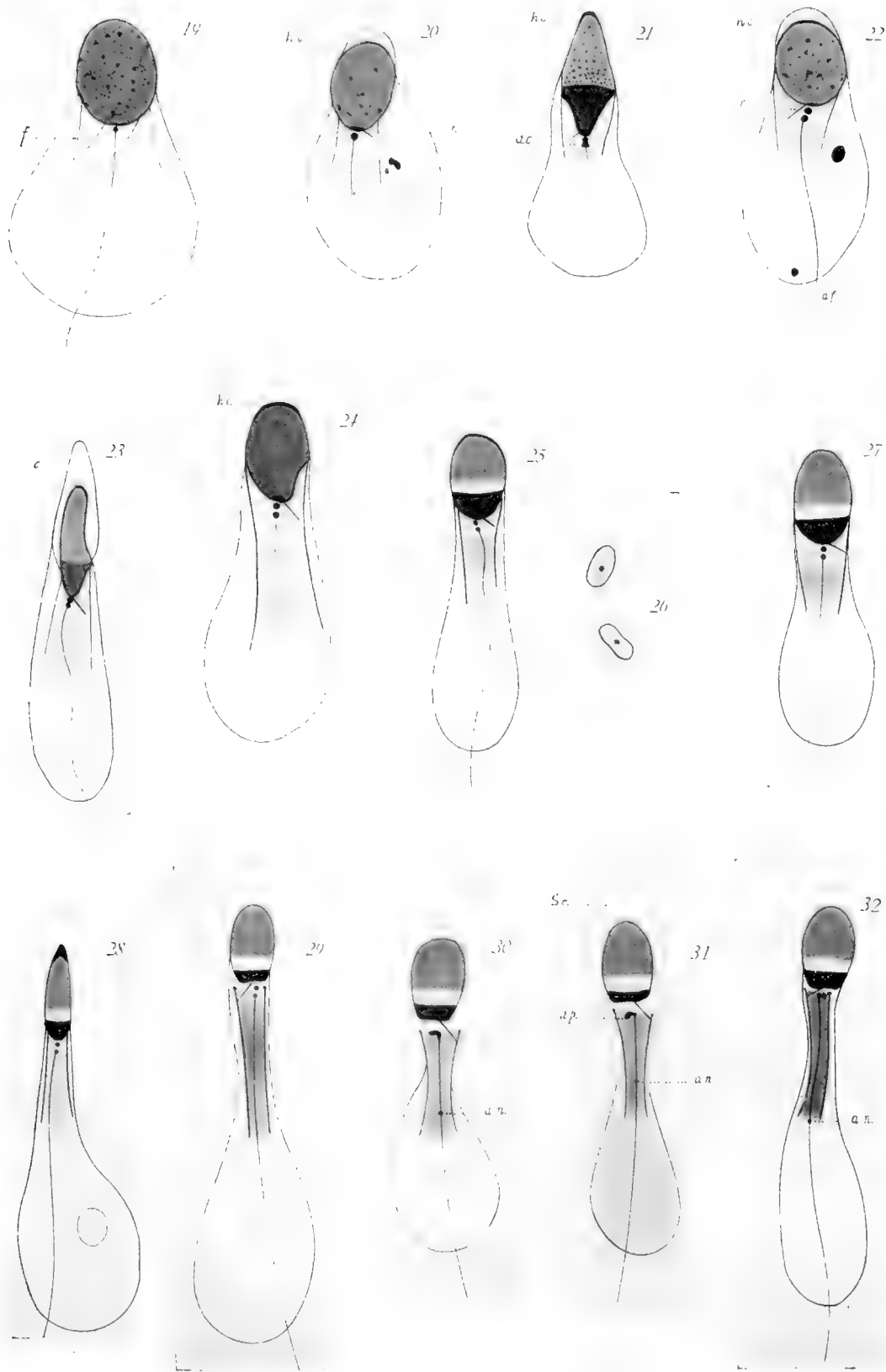
Fig. 29 The spermatid is still more elongate, the caudal tube has become separated from the nucleus, and is beginning to narrow distally, its contents staining more deeply. Its wall shows a nodule at the anterior end, the cross section of the thickened margin.

Figs. 30 to 32 and 34 to 35 Third period of spermiogenesis.

Fig. 30 The separation of the caudal tube from the nucleus has about reached its maximum. The proximal centriole is flat, its rodlet is in contact with the caudal tube. The posterior portion of the distal centriole has wandered down the axial filament, and now forms the annulus, *an.* The anterior portion of the distal centriole has elongated and bent at right angles with itself, thus forming a transverse and a vertical limb. The cell membrane fuses with the wall of the caudal tube about midway of its length.

Fig. 31 A spermatid imbedded in the cytoplasmic prolongation of a Sertoli cell, *S.c.*, which is shown only in part. As is usually the case the outline of the cell membrane of the spermatid may be traced readily in its distal portion, but becomes lost when surrounded by the cytoplasm of the Sertoli cell. The outlines of the caudal tube are sharply defined. The annulus, *an.*, is migrating down the axial filament, and the anterior portion, *n.p.*, of the distal centriole is in the act of dividing into two unequal portions.

Fig. 32 The annulus, *an.*, has now reached the posterior end of the caudal tube, the limit of its migration. The two bodies derived from the division of the proximal half of the distal centriole in figure 31, now lie side by side at the anterior end of the caudal tube. The shrinkage of the latter is in progress and the tube is narrowing rapidly. A very characteristic displacement to the left is seen, due apparently to the resistance of the rodlet, which is in contact with the tube at its anterior end.



Figs. 33, 36, 37, 38 Fourth period of spermiogenesis

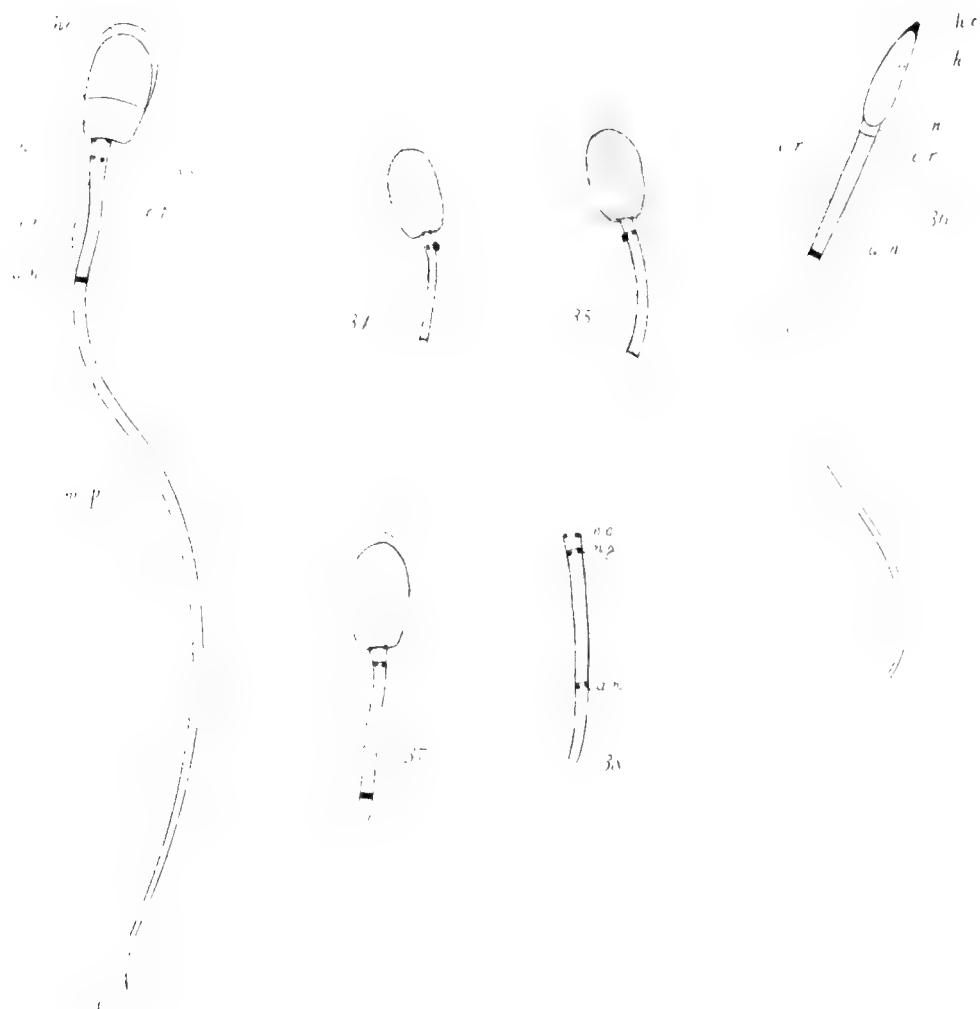
Fig. 33 Nearly mature spermatozoon from the epididymis. Face view. The whole structure now stains much lighter than before. The head is covered by the head cap, *h.c.*, the posterior margin of which forms a sharp slightly curved line. The posterior end of the head stains darker and immediately in front of this region is a lighter zone. The neck, *n*, is clear and shows the two anterior centrioles, Noduli anteriores, in front in contact with the head. The connecting piece, pars conjunctionis, *c.p.*, still bears a small cytoplasmic remnant, *c.r.* It is limited anteriorly by the two Noduli posteriores, now equal in size, and posteriorly by the annulus, *an.*, still visible as a flattened disc. Neither the connecting piece nor the main piece, pars principalis, *m.p.*, show the axial filament in this preparation. The main piece tapers posteriorly to the very slender end piece, pars terminalis, *e.p.*

Figs. 34 and 35 Anterior portion of two nearly mature spermatozoa from a tubule. The cytoplasmic remnant still adheres to the anterior end of the connecting piece. The inequality of the two Noduli posteriores is very characteristic at this stage.

Fig. 36 Profile view of nearly mature spermatozoon. The head cap is stained deeply. Lettering as in figure 33. The tail is incomplete.

Fig. 37 Head of mature spermatozoon from epididymis, showing exceptionally symmetrical insertion of the tail.

Fig. 38 Neck, connecting piece and a portion of the main piece of the tail of a mature spermatozoon from the epididymis. The head has been broken off, leaving the two anterior centrioles, *n.a.*, in the neck.



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